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MANGANESE AS AN ESSENTIAL ELEMENT FOR PLANT GROWTH

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(With Plates XXII–XXIV and 2 Text-figures.)

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I. INTRODUCTION.

In a previous paper (Samuel and Piper, 1928) it was shown that the Grey Speck disease of oats, which occurs on certain types of soil, is a manganese deficiency disease. A certain amount of evidence was also given that it is rather a lack of *available* manganese in the soil than actual manganese deficiency which is responsible. Soils which do not appear to have sufficient available manganese for the growth of oats (and to a less extent of barley and wheat), nevertheless support an apparently normal growth of pasture plants and weeds. The question is therefore raised as to the necessity of manganese for such pasture plants; and, if it is necessary, whether it is that they require much smaller quantities for normal development than do the cereals, or whether they are able to absorb more of what manganese is present than can the cereals. (It may be noted here that rye is an exception among the cereals, and will grow well on land where oats suffer severely from manganese deficiency.)

Of recent years, mainly owing to the work of McHargue (1922, 1926), it has come to be recognised that manganese is an essential element for plant growth. McHargue showed, by sand and water culture experiments, that a number of plants could not be grown to the fruiting stage in the absence of manganese. With wheat in water-cultures he obtained an increase of 135 per cent. in dry weight in the plants supplied with manganese as compared with those deprived of this element, and with peas an increase of 67 per cent.

In the present investigation it has been found that when careful precautions are taken to exclude all traces of manganese, the increases in dry weight in different plants to be expected from the addition of manganese range from 300 to over 5000 per cent. Except in the case of rye, the increases for the cereals and grasses used all ranged from 1000 to 5000 per cent.

Such striking results as these were not obtained by McHargue, and it would appear probable that traces of manganese must still have been available to the plants in his experiments which he considered to be growing in solutions free from manganese. That such results would probably be obtained if extra care was taken in the purification of chemicals and exclusion of manganese was forecasted, however, by Sommer and Lipman (1926), who obtained similar results in their experiments on the necessity of zinc and boron for the growth of higher plants.

Historical.

Published work on the relation of manganese to plant growth is very voluminous. A review of much of the earlier work is given by Brenchley (1927).

Bertrand, led on to the subject from his work on the relation of manganese to laccase, has been insisting for nearly thirty years on the essential nature of manganese in the plant economy. It was the universal presence of manganese in plant ash, and its beneficial effect as a fertiliser, as well as his experiments on its relation to oxidases, which led him to this opinion. He did not support it with definite culture experiments with precautions to exclude manganese, except in the case of the fungus *Aspergillus niger*, which he showed would not form conidia in the absence of this element.

The work of Bertrand, together with that of investigators in Japan (Loew, Aso, Sawa and others) on the stimulating action of manganese salts on rice and other plants, started a vogue for so-called "catalytic" fertilisers, among which manganese was perhaps considered the most important. This was reflected in the publication of the results of numerous empirical fertiliser tests on all kinds of agricultural crops, using the salts of manganese and other rarer elements.

Many of these tests showed appreciable increases in crop yield as a result of the use of fertilisers containing manganese, but others provided only doubtful or negative evidence of their value. Very few of these publications contained any information whatever upon either the manganese content or the reaction of the soil upon which the experiments were tried.

The earlier water or sand-culture work was concerned more with the toxicity of manganese, which may be evident in dilutions as low as 1 : 1,000,000, than with its essential nature, although a "stimulant" action was noted at high dilutions by Aso as early as 1902. It was not until 1914 that Mazé began to investigate the problem of the necessity of traces of the rarer elements by means of water-cultures with carefully purified salts. He demonstrated that manganese, as well as a number of other rarer elements, was necessary for the growth of maize. McHargue (1922) later proved by sand and water-culture experiments that manganese was necessary for the growth of a considerable number of plants. Schreiner and Dawson (1927) and Miller (1928) have confirmed this in pot experiments with tomatoes and certain other plants. Bishop (1928) also found manganese necessary in sand cultures with maize, peas, beans

and radishes. The necessity of manganese for normal growth is now recognised to such an extent that it is included as a matter of course in a complete nutrient solution for water-culture work such as that of Sommer and Lipman (1926) and Sommer (1928).

The *symptoms* developed by plants suffering from manganese deficiency have been described by all the above authors as a chlorosis. McHargue (1922) states, "the first effect to be noted in the growth of plants from which manganese has been withheld is a lack in the development of chlorophyll in the newly formed tissues or in the growing parts of the plant. This condition increases with time, and finally results in the tips of the branches dying back and a cessation of further growth of any consequence in the plant."

Schreiner and Dawson (1927), describing the symptoms of manganese deficiency in tomatoes, say, "the chlorosis manifests itself at first as a lightening of the green colour, turning to yellow, in the leaf areas farthest from the major veins. As the condition progresses the yellow becomes more marked and extensive, the veins still remaining green, giving a characteristic mottled appearance to the leaf. Eventually the foliage may become completely yellow; and in many cases, especially on the untreated soil, a necrosis sets in, appearing at first as tiny brown pinpoints centring in the yellow areas farthest from the veins and expanding to larger dead areas indicating complete breakdown of the tissues."

Miller (1928) also describes the effect as a "chlorosis which is apparently quite characteristic, being chiefly recognised by the fact that in most plants the yellow colour is located in areas away from the veins, thus producing a mottled appearance, or in the case of grasses, a striped effect."

It would appear from these quotations that the symptoms of manganese deficiency are not those of a normal chlorosis in the usual sense of the word, such as results from iron deficiency. This is a point which does not seem to have been sufficiently emphasised in the past, and it is further discussed below.

With regard to the function of manganese in plants, although a number of theories have been advanced, none of them is supported by sufficient experimental work to make any one of them conclusive. Bertrand considered that manganese was an essential part of the oxidase system of plants. McHargue (1922) claimed that manganese performs an important function in carbon assimilation and the synthesis of

chlorophyll, and later (1924) drew attention to an apparent correlation between the occurrence of manganese and vitamins.

Naturally occurring manganese deficiency diseases.

For a long time the Grey Speck disease of oats seems to have been the only manganese deficiency disease occurring in the field for which it was recognised that an application of a soluble manganese salt was the best cure (Riehm, 1917). At the same time it does not seem to have been recognised that the symptoms of the Grey Speck disease are essentially the symptoms of manganese deficiency until this was proved by the present writers (1928). It was known that the trouble appeared on certain types of soil, from nearly neutral to fairly strongly alkaline in reaction, but very different theories were advanced as to the cause of the trouble. Hudig (1923) considered that small amounts of certain organic substances, in conjunction with the alkaline reaction, were responsible. Arrhenius (1923 and 1924) considered that the disease was always associated with an excess of calcium ions in the soil solution. Hiltner (1924) believed that the beneficial effects of manganese were due to an indirect effect on the carbonic acid assimilation of the plants. He formulated a "Carbonic-acid mineral-substance Law," and advanced the proposition that under conditions unfavourable for adequate carbon assimilation the stimulatory action of manganese enables the plants to assimilate their food supply and make full use of the nutritive elements supplied by the soil.

The next naturally occurring manganese deficiency disease for which it was recognised that an application of a manganese salt was a cure was the chlorosis of spinach described by McLean and Gilbert (1925). At this time, however, the importance of manganese as a plant nutrient was being emphasised by McHargue, and it was realised from the commencement that the spinach trouble was definitely a result of manganese deficiency (Gilbert and McLean, 1928).

Later Schreiner and Dawson (1927) showed that failure of tomatoes in certain highly calcareous glade soils could be corrected by an application of sulphate of manganese.

Willis (1928) showed that oats and soy beans suffered from manganese deficiency on some coastal plain soils of North Carolina.

Finally, Lee and McHargue (1928) have demonstrated that Pahala blight of sugar cane is a manganese deficiency disease, giving chemical analyses as well as demonstrating that the application of manganous sulphate would cure the trouble.

II. EXPERIMENTAL WORK.

The experimental work aimed at investigating the essential nature of manganese for plant growth by means of water-cultures with greater precautions than have been observed hitherto; and at determining factors influencing the absorption of manganese, by means of water-cultures and chemical determinations of the manganese present in experimental and in field plants.

The special precautions observed in the water-culture work, including preparation of a manganese-free iron salt, and paraffining of all glass surfaces in contact with nutrient solutions, are detailed in Appendix I.

A. Plants tested in water-culture for the essential nature of manganese.

Table I gives a list of twenty plants which were grown in water-culture to determine whether manganese was essential for their normal development.

Table I.

Showing the dry weights of plants grown in water-cultures with manganese and without manganese.

	Dry weight in gm.	
	With manganese 1 : 1,000,000	Without manganese
Wheat: Federation ...	*	*
Sepoy	150	14
Late Gluyas ...	149	6
Oats: Algerian	185	8
Lachlan	*	*
Mortgage Lifter ...	*	*
Imbros Island ...	*	*
Barley: Cape	101	6
Rye	86	27
Danthonia penicillata ...	47	0
Lolium subulatum	131	19
Phalaris bulbosa	114	0
Bromus unioloides	109	4
Peas	46	11
Broad bean	87	26
Lucerne	20†	7
Medicago denticulata ...	17†	4
Trifolium subterraneum ...	48†	5
Tomato: Dwarf Red ...	—	—
Maize	158	29

* Varieties not carried on after manganese deficiency symptoms established with certainty.

† Changed after six weeks to Mn 1 : 5,000,000 solution, since Mn 1 : 1,000,000 appeared toxic.

Four culture jars of each variety, each containing six seedlings (except in the case of broad bean, where there were only two seedlings), were prepared with manganese-free culture solution. To two of these jars 10 c.c. of 128 per cent. pure manganese sulphate ($MnSO_4.4H_2O$) were added, sufficient to make the concentration of manganese in the solution one part in a million.

Boric acid (1 : 2,500,000) was added to all jars containing leguminous plants.

Losses due to transpiration were replaced with distilled water, and the solutions were changed twice during the growing period.

In many cases the seedlings in the two manganese-free jars were either dead or almost dead before the first change of solution, about ten weeks after commencement. Those in the solutions containing manganese grew vigorously and healthily at all times. Occasionally plants became somewhat yellowish as if needing more iron, and in these cases an extra half dose of ferric citrate was given to all four jars of a series, even if only one jar was showing the need of iron.

The cultures were kept going until the plants in jars containing manganese had passed the flowering stage, and in some cases had formed ripe seed (Plate XXIII, fig. 1). They were then harvested and the dry weights determined. (Varieties of oats other than Algerian were not carried on after pronounced symptoms of manganese deficiency had manifested themselves on all the seedlings in manganese-free solutions.)

The effect of withholding manganese from the plants can be well seen in the figures in Plates XXII and XXIII. None of the plants tested has been able to develop beyond the seedling stage in the absence of this element. This fact is reflected in the comparative dry weights of the cultures (Table I) which show increases ranging from 300 to over 5000 per cent., due to the addition of one part of manganese (as sulphate) to a million of culture solution. There is little doubt that the lower figures would also have been considerably increased if the best culture conditions had been known from the start. The clovers, for example, did not grow well in the jars containing manganese 1 : 1,000,000 and boric acid 1 : 2,500,000, but when the concentrations of each were reduced to 1 : 5,000,000 they grew ahead normally.

Symptoms of manganese deficiency.

In general it may be said that all plants germinated and grew healthily for a few weeks, presumably with the aid of the manganese stored in the seed. The amount of healthy growth made in manganese-free solutions

was in some relation to the size of the seed. Peas and beans grew to nine inches or more in height before cessation of growth, whereas cereals usually showed the deficiency about the time of tillering, when they were from three to five inches high, and some grasses with small seeds scarcely grew at all in the manganese-free solutions.

In all cases the onset of manganese deficiency symptoms was comparatively sudden.

Peas. With peas, for example, the growth in the manganese-free solutions appeared just as healthy and vigorous as that in the solutions containing manganese for 38 days from the date of germination. Growth

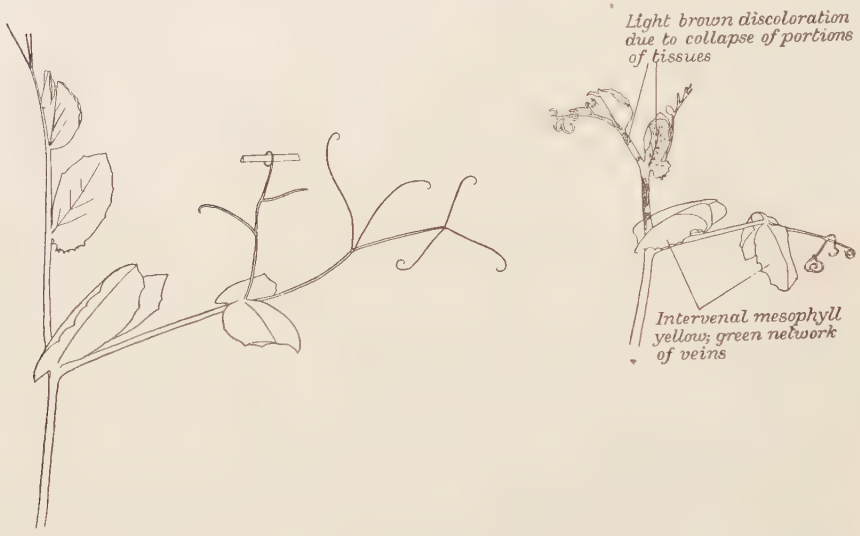

Fig. 1. The growing points of two plants of Brunswick White Peas; that on the left from water-culture containing nutrient solution plus manganese 1 : 1,000,000, and that on the right from water-culture containing nutrient solution free from manganese.

of the plants in the manganese-free solutions then ceased, young tendrils and the youngest internodes at the top of the stem acquired a brownish discoloration, at first superficial, and the youngest leaves failed to expand, becoming yellowish with small discoloured areas between the veins where the mesophyll had collapsed. Slightly older, but not fully expanded, leaves acquired a characteristic mottled appearance due to the mesophyll between the small veins becoming yellow, the veins themselves remaining green and forming a fine green network over the leaf with meshes of yellow about 1 mm. in diameter. All the lower fully formed leaves retained their normal green colour. The changes at the

growing point were pronounced within two or three days after the symptoms were first noticeable, and the plants in the solutions containing manganese offered a striking contrast, being already several inches higher and with normal healthy green growing tips (Fig. 1). No further growth took place in the solutions free from manganese, the growing tip and the youngest leaves being completely dead in a fortnight. The lower part of the plants remained alive for months. (Plate XXII, fig. 1.)

Beans. Beans also ceased growth at the growing points after seven or eight weeks, the young undeveloped leaves becoming pale coloured and flecked with brownish intervenal spots, but the lower part of the plants remaining healthy for many weeks. (Plate XXII, fig. 2.)

Clovers. Clovers grew well for about eight weeks, and had formed short horizontal stems before symptoms of manganese deficiency were observed. It was then noted that the tips of the stems ceased growth, the young leaves became somewhat yellowish and developed brown flecks upon them, and later died from the margins inwards. The further development of the plants was checked owing to the collapse of the growing points of the stems and the apparent inability to develop further stems. Finally the plants in the manganese-free jars died completely. (Plate XXII, fig. 3.)

Oats. With Algerian oats the first symptoms of manganese deficiency in water-cultures in the glass-house appeared about four weeks (sometimes less) from the date of germination. The symptoms were a discoloration and collapse of the tissues of the leaf-blade in a characteristic manner. The trouble was usually first visible in a thin strip at each edge of the blade at a position about an inch from the leaf-base, and soon extended across the blade so that the tip three-quarters of the leaf fell over with a sharp kink at the collapsed portion. The first formed leaf was never affected in this manner, but the second, third and later leaves became affected successively, the distal end of the leaves remaining green for a considerable time after the lower portion had collapsed. (Plate XXIV, fig. 1.) On older leaves the collapse was not so markedly confined to the lower quarter and oval spots of collapsed tissue appeared irregularly on the leaf-blade, though less frequently towards the tip end. Streaks of tissue collapsing at the margins of the leaves were also very characteristic. (Plate XXIV, figs. 2 and 3.)

Finally new leaves ceased to push up through the leaf-base of the uppermost leaf, indicating death of the growing point, and all leaves shrivelled gradually and the plant completely died. (Plate XXIII, fig. 2.) It was pointed out in a previous communication (1928) how these

symptoms of manganese deficiency in water-cultures corresponded exactly with the symptoms of Grey Speck disease of oats in the field.

The symptoms described above were those observed when oats were grown in a culture solution completely free from manganese. When manganese sulphate was added to make the concentration of manganese one part in fifty million, the amount proved to be inadequate for the growth of six seedlings to maturity without change of solution, but healthy growth and abundant tillering occurred for the first nine weeks. After this the characteristic marginal strips and spots of collapsed tissue, situated mainly on the basal half of the younger leaves, again indicated manganese deficiency. No symptoms of chlorosis, in the sense of yellowing of leaves, were at any time observed in connection with manganese deficiency of oats.

Wheat and barley did not show such characteristic symptoms as oats. The first symptoms were not noticed until a somewhat later stage, when three or four short tillers had been formed and the plants were five or six inches high. Streaks of collapsed tissue appeared on the younger leaves, running especially between the veins, and again situated mainly on the basal half of the leaf. In some cases these intervenal streaks were whitish, due to collapse of the mesophyll tissue. These whitish intervenal streaks were much more pronounced on the leaves of *maize* plants in manganese-free solutions, and closely correspond with those figured by Lee and McHargue (1928) for Pahala blight, or manganese deficiency, of sugar cane. Few new leaves were formed in any of these cereals after the appearance of deficiency symptoms, those which did grow out soon succumbing in a similar manner, and the plants remained dwarf for some weeks and ultimately gradually died.

Grasses. Grasses in most cases died at such an early stage in manganese-free solutions that the detailed record of symptoms was difficult. (Plate XXII, fig. 4, and Plate XXIII, fig. 4.) However, in the seedlings which attained a small size similar symptoms were noted to those described for the cereals. Irregular patches of collapsed tissue, frequently marginal, or intervenal streaks of collapsed tissue, appeared along the leaf-blades, followed by cessation of growth and later death.

Tomatoes. Tomatoes behaved somewhat differently in that the growing points of the plants in manganese-free solutions did not definitely die as in other cases, but the plants remained very dwarf, spindly, and almost stationary at about four inches high. The leaves formed showed a characteristic intervenal chlorosis as described and figured by Schreiner and Dawson (1927) and by Miller (1928).

It will be seen from the above that the general symptoms of manganese deficiency are a sudden cessation of growth with collapse of portions of the immature tissues, and gradual death of the growing point while the lower part of the plant remains alive, green, and apparently healthy for weeks. In dicotyledons a poor development of chlorophyll in the immature tissues gives a chlorotic appearance to the growing point. Leaves not quite fully developed frequently acquire a characteristic appearance owing to the intervenal mesophyll becoming yellow and leaving the veins standing out as a network of green.

In cereals and grasses the leaf symptoms are the more evident, owing to the growing point of the stem not being visible. Long whitish intervenal streaks in which the chlorophyll is not developed as in maize, or definite irregular patches of collapsed mesophyll tissue as in oats, are the two main types of symptoms to be observed.

It is desired to emphasise the fact that these symptoms are much more than a chlorosis in the usual sense of the word, and they cannot possibly be confused with chlorosis due to lack of iron (see the section on *Symptoms* in the Introduction). In other words, the effect of manganese deficiency is essentially an effect on the immature tissues at the growing point, although non-development of chlorophyll in these tissues may also be a characteristic.

The anatomical changes of which these symptoms are the expression will be described in detail in a further communication.

B. EXPERIMENTS WITH OATS.

Oats were chosen for a more extended series of water-culture and pot experiments on account of the characteristic symptoms of manganese deficiency which they exhibit. This is a very great advantage, since with the majority of deficiency diseases the symptoms cannot be considered so specific that the deficiency can be named at a glance. With oats, moreover, the symptoms appear early—in four weeks or less from the date of germination if the nutrient solution is free from manganese. And also the manganese requirement of oats appears to be fairly high in comparison with that of some other plants, as will appear from the following experiments.

(a) *The non-replaceability of manganese with other elements.*

To each of ten jars containing the manganese-free nutrient solution a different element was added as shown below, and control jars with and without manganese completed the series.

(1)	+B	1 : 2,500,000	as H_3BO_4	(7)	+Ba	1 : 2,500,000	as $BaCl_2$
(2)	+Al	,,	,, $Al_2(SO_4)_3$	(8)	+Sr	,,	,, $Sr(NO_3)_2$
(3)	+Zn	,,	,, $ZnSO_4$	(9)	+I	,,	,, KI
(4)	+Cu	,,	,, $CuSO_4$	(10)	+Si	,,	,, K_2SiO_3
(5)	+Co	,,	,, $Co(NO_3)_2$	(11)	+Mn	,,	,, $MnSO_4$
(6)	+Ni	,,	,, $Ni(NO_3)_2$	(12)	Nutrient solution alone		

Six plants were grown per jar. Within from six to seven weeks the plants in all jars except that containing manganese were showing the typical dying of the leaves characteristic of manganese deficiency as exhibited in jar 12. The series was discarded after twelve weeks' growth, when the plants in all jars were rapidly dying back from manganese deficiency except those in jar 11 containing manganese sulphate, which were vigorous and healthy.

In another series a combination of the elements zinc, copper, boron and aluminium (each in dilution 1 : 10,000,000) was used. Four jars were prepared with these elements added to the manganese-free culture solution, and to two of them manganese 1 : 5,000,000 was added in addition. The seedlings in the two jars with Zn, Cu, B, and Al, but without manganese, developed symptoms of manganese deficiency in about four weeks, whereas those in the jars containing manganese grew perfectly normally.

(b) *The effect of different concentrations of manganese in the nutrient solution.*

Several investigators (Aso, Brenchley (1927) *et al.*) have determined the upper limits of concentration of manganese for barley and peas in water-culture. In dealing with the lower limits they have only reported a "stimulant" action observable at high dilutions. If manganese is an essential element as proved above, this stimulative effect is probably only due to a sufficiency of the element having been supplied for maximum growth under the existing conditions, there having been an insufficiency in the normal culture solution.

To investigate the lower limits with a culture solution known to be absolutely manganese-free, the following series with oats was therefore arranged (in triplicate):

Mn-free,
Mn 1 : 50,000,000,
Mn 1 : 10,000,000,
Mn 1 : 5,000,000,
Mn 1 : 1,000,000.

The plants in the manganese-free jars were showing symptoms of manganese deficiency within four weeks, and were never able to grow more than a few inches high. Those in the jars with manganese 1 : 50,000,000 grew well and tillered as freely as any of the plants in solutions of higher manganese content until about eight weeks old, when they suddenly developed symptoms of manganese deficiency. None of the other plants showed evidence of lack of manganese at the end of ten weeks' growth, at which time the solutions were changed to supply sufficient of the general nutrient salts for continued growth. The change of solution, with its fresh supply of manganese, permitted the plants in the 1: 50,000,000 jars to shoot ahead again, and fresh healthy leaves appeared which developed no further deficiency symptoms until some four weeks later. Soon after this, however, the second change of solution again permitted further healthy growth, and at the time of harvesting these plants were nearly three feet high and producing a few ears. No symptoms of manganese deficiency were at any time observed on the plants in solutions with 1 : 10,000,000 of manganese or more. The dry-weights of the whole series when harvested were 14, 118, 241, 270 and 257 gm. respectively for the various concentrations. These figures would indicate that concentrations of one part of manganese in from one to five million parts of solution are the optimum for the growth of oats in water-cultures in which the solutions are changed only once or twice during the growing period. It would appear probable, however, that if the solutions were changed more frequently one part of manganese in ten million parts of solution would give as good a growth, and that if a method of continuous solution change was arranged, oats might be grown to maturity in a culture solution containing as little as one part of manganese in fifty million parts or more of solution.

(c) *The effect of removing manganese at different stages of growth.*

Twelve jars of oat seedlings were started in the nutrient solution with addition of manganese 1 : 500,000. After four weeks the plants in three jars were removed, their roots washed with distilled water, and the plants replaced in a manganese-free culture solution. Three more jars were similarly removed to a manganese-free solution after six weeks, and three more after eight weeks, the remaining three being grown on continuously in the manganese-containing solution, with one change of solution after ten weeks' growth.

The plants removed to manganese-free solution after four weeks' growth showed no evidence of manganese deficiency until they were ten

to eleven weeks old. These plants managed to grow on, showing symptoms of manganese deficiency on all the upper leaves, and finally formed some heads at about 2 ft. 6 in. high. The plants had fewer tillers and were 6–9 in. shorter than normal plants.

It is probable that the amount of manganese these plants were able to absorb during their first four weeks' growth was just insufficient to enable them to reach maturity in a normal healthy manner. This point is further discussed in section C (c), dealing with the minimum quantity of manganese necessary for complete development.

The plants removed to manganese-free solutions after six and eight weeks' growth, and those grown continuously in the solution containing manganese, at no time showed evidence of deficiency and grew to a height of over three feet and flowered well.

(d) *Comparison of oats and rye as to manganese requirement.*

The fact that the strip of rye sown along the roadside by the farmers of Mount Gambier develops to maturity where oats would fail, makes it interesting to determine whether this is due to a lesser manganese requirement on the part of rye, or whether it is due to a greater absorption of manganese by this crop under the existing conditions.

The former possibility was capable of test by water-cultures. It was known from a previous experiment that the amount of manganese supplied in a water-culture in which the concentration of this element was 1 : 50,000,000 was insufficient for the normal development of six oat seedlings. Accordingly two jars each of Algerian oats and rye were set up, each containing the standard nutrient solution plus manganese 1 : 50,000,000, with four seedlings per jar.

After several weeks' growth the oats developed pronounced symptoms of manganese deficiency, the leaves dying in the typical manner, no young leaves appearing, and the plants never attaining a height of more than one foot. They were not able to produce flowering heads. The rye, on the other hand, progressed well, showed no symptoms of manganese deficiency, and came into flower at a height of over two feet.

Further work is now being undertaken to determine with more precision the relative requirements for manganese of certain plants. The above experiment gives very suggestive indications, as does also the table of chemical determinations of manganese in plants from manganese-deficient soils (Table VI).

C. CHEMICAL DETERMINATIONS OF THE MANGANESE CONTENT OF OATS GROWN UNDER DIFFERENT CONDITIONS.

The above series of water-culture experiments raise interesting questions as to the manganese content of oat plants, its variability, and the possibility of there being an amount of manganese which must be regarded as the minimum quantity essential to support healthy growth to maturity. The following three sections provide information gained upon these points by systematic chemical work on oat plants from the field and from the water-cultures. The method used for the chemical determination of manganese is given in Appendix II.

(a) *Variation in manganese content with growth (oats and barley).*

In order to follow the changes in manganese content of oat plants during their period of growth under natural conditions, duplicate samples were taken at intervals of ten days throughout the growing period from a plot of Algerian oats in the Waite Institute Experimental Field. The plot was about 100 yards long, and each sample was obtained by cutting several plants at intervals of about ten yards along the length of the plot, and bulking these for analysis. The duplicate samples, taken adjacent to one another at each sampling, were always in good agreement.

The plot was sown on June 8th, 1928, 65 lb. of seed and 92 lb. of superphosphate being used per acre. The oats made slow growth at the start and the first sample was not collected until August 8th. The flowering stage was reached on October 17th and the plot was harvested on November 29th. The grain and straw samples were collected during harvesting.

The plot yielded at the rate of 4 tons 2 cwt. total produce, and 81·2 bushels of grain per acre.

Table II shows the percentage of crude ash and the amount of manganese expressed as parts per million parts of dry matter. The figures given are the average of the duplicate samples except that for October 17th which is the average value of 32 individual plants taken on this day.

The most noticeable feature of the manganese determinations is the increase in the proportion of this element between about the twelfth and sixteenth weeks of growth. From then there was a steady decline until the plant was ripe, but even then the proportion of manganese is only a little less than at the time of the first sampling.

Table II.

Showing the ash and manganese content of oats at different stages of growth.

Date sampled (1928)		% Ash on dry matter	Mn p.p.m.
(Sown)	June 8th	—	—
	Aug. 8th	11·59	71·2
	18th	11·30	58·5
	28th	10·25	70·5
	Sept. 7th	10·83	68·8
	17th	10·46	80·1
	27th	10·38	92·4
	Oct. 8th	8·95	91·7
(Flowering)	17th	7·56	81·1
	27th	7·38	82·7
	Nov. 6th	6·91	76·6
(Harvested)	29th (whole plant)	6·17	63·8
	29th (grain)	4·03	62·7
	29th (straw)	7·32	65·7

It remains to be determined whether these figures will show much seasonal variation. The average manganese content of three samples of Algerian oats taken from different plots at the Waite Institute during 1927 was 67·3 p.p.m., the samples being collected ten to twelve weeks after seeding. This agrees with the amount found at the same stage during 1928. But the manganese content of oat grain has varied from 40·3 p.p.m. in 1927 and 50·9 p.p.m. in 1926 to 62·7 p.p.m. in 1928. Similarly the manganese content of ripe plants of Algerian oats was only 22·4 p.p.m. in 1927 as against 63·8 p.p.m. in 1928.

Some samples of barley grown in pots during 1926 and harvested at different stages of growth were available and the manganese was determined in these. The amount found is shown in Table III.

Table III.

Showing the ash and manganese content of barley at different stages of growth.

Date sampled (1926)	% Ash on dry matter	Mn p.p.m.
Sown: May 12th	—	—
July 19th	17·70	57·3
Aug. 9th	17·68	41·7
Aug. 30th	15·00	40·7
Sept. 20th	12·17	33·9
Oct. 11th	9·23	26·1
Nov. 9th	8·88	26·4

It is seen that in this case there was a continuous decrease in the proportion of manganese to dry matter as the plants approached maturity. Whether such a marked decrease would also have been found in plants taken from the field is as yet uncertain.

(b) *The variability in manganese content of individual oat plants.*

The plot of Algerian oats mentioned in the preceding section was sampled at the flowering stage, by cutting 25 plants spaced at approximately equal intervals along its length. The individual plants were kept separate and analysed for ash and manganese to determine the variation

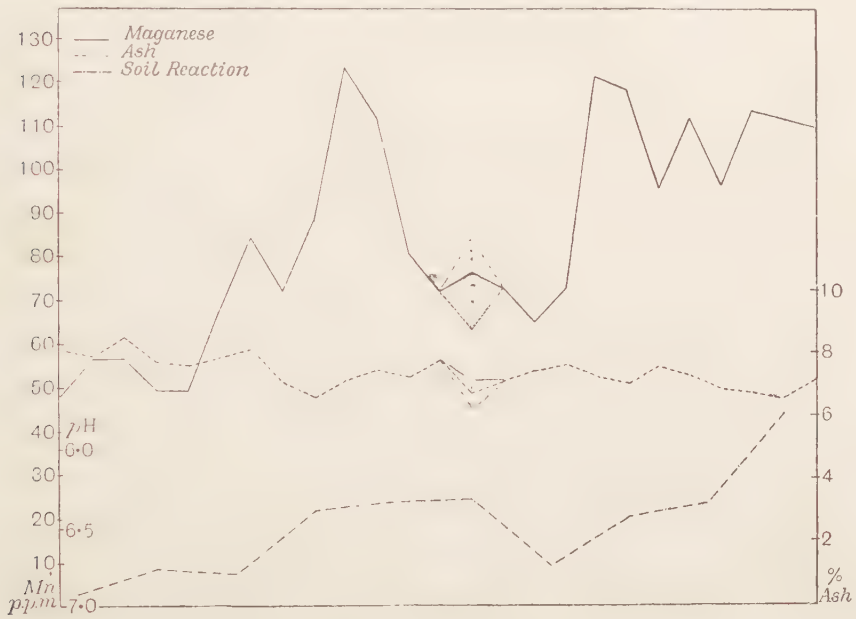

Fig. 2. Graph showing the variation in manganese and ash in 25 individual oat plants (variety Algerian) taken at approximately equal intervals along a field plot. In the centre the variation for eight plants taken from a small area about eight inches square is shown. The variation in soil reaction along the plot (taken some months later) is also indicated.

from plant to plant throughout the plot. Near the centre eight plants were taken from a small area about eight inches square to determine the natural variation in plants growing at the same spot. The manganese content of these eight plants growing alongside each other varied from 63·0 to 84·2 parts of manganese per million parts of dry matter and

averaged 76·2 p.p.m. From the separate values the probable error of any single determination was calculated and found to be 6 per cent. This may be taken as the probable error of the other single plant samples.

The results of all the determinations are shown diagrammatically in Fig. 2, the amounts of manganese and ash in the individual plants being plotted in relation to the approximate position of the plant in the plot.

It will be seen that there is a progressive variation in manganese content throughout the plot. The first few samples are relatively low in manganese. There is then an increase reaching a maximum in the tenth sample. Near the centre of the plot the amount of manganese is definitely lower but not as low as in the first five samples. The last eight plants are again much richer in manganese.

The large variation in the manganese content throughout the whole plot (48·3–123·7 p.p.m.) is believed to be mainly due to two factors, namely the soil reaction and the flooding of the plot by some of the winter rains. Unfortunately soil samples to determine the reaction were not taken until some months after the plant samples, and so it was not possible to take them from the exact spot on which the plant was growing. Ten samples were taken at approximately equal distances along the plot and the *p*H determined by the quinhydrone electrode, the values found being shown in the diagram (Fig. 2).

It will be seen that considerable variation in soil reaction occurs from point to point along the plot, the maximum difference found being 1·1 *p*H units. There is some correlation between the curves of the manganese content of the plant and the soil reaction, the higher manganese figures being associated with the more acid soil conditions.

However, the uneven flooding of the plot by some of the winter rains and the resultant water-logging of the soil have had an effect which it is difficult to evaluate. Further investigations, in which the soil and plant samples are taken at the same time and from the same place, are needed to establish the correlation between soil reaction and the manganese content of the plant.

Owing to the variation in soil reaction found in the apparently uniform plot investigated, it would appear that in taking plant samples for manganese determinations from ten to twenty plants should be cut from an area of a few square yards, similar samples being taken from at least two other places in the field. A sample of soil should also be taken from each place and the reaction determined.

(c) *The minimum amount of manganese found in healthy oat plants at the flowering stage.*

From the great variation in the manganese content of healthy oats grown at different places, it appears that in a large number of cases there must be far more manganese absorbed than is actually needed for normal growth. It was thought that the analysis of the tops of oats grown in the water-cultures with the smaller concentrations of manganese would give an indication of the minimum amount of that element to be expected in healthy plants. Table IV shows the manganese content of some of the oats grown in water-cultures during 1927 and 1928. At the time of harvesting, the 1928 series was at the flowering stage and the 1927 series was somewhat more advanced. In each case the oats grown in the manganese-free nutrient solutions had died at much earlier stages.

Table IV.

Showing the manganese content of Algerian oats from water-cultures.

Year	No.	Mn in nutrient solution	Mn in plants p.p.m. dry matter	Mean p.p.m.	Severity of Mn deficiency symptoms
1927	A 1–4 and C 2–4	Nil	—	6·2	Died as seedlings
1928	B 1–3	Nil	—	12·3	,,
	B 4	1 : 50,000,000	7·7	8·5	Badly diseased
	5		8·9		
	6		9·0		
	F 1	*	12·3	12·1	Diseased
	2		11·3		
	3		12·7		
	B 7	1 : 10,000,000	14·05	14·8	Healthy
	8		13·9		
	9		16·35		
	B 10	1 : 5,000,000	21·4	21·3	,,
	11		19·0		
	12		23·5		
1927	C 5	1 : 4,000,000	18·3	21·0	,,
	6		23·9		
	7		18·0		
	8		23·6		
1928	B 13	1 : 1,000,000	73·4	82·9	,,
	14		82·5		
	15		92·9		
1927	C 9	1 : 400,000	111	95·7	,,
	10		103		
	11		85·2		
	12		83·7		

* Plants in Mn 1 : 500,000 for 1 month and then transferred to Mn-free nutrient solutions.

From the foregoing results it is seen that the oats which died at an early stage of growth from lack of manganese contained 6·2–12·3 parts of manganese per million parts of dry matter. Of the plants which exhibited characteristic symptoms of the disease but were still alive at harvesting (B 4–6 and F 1–3) none contained more than 12·7 p.p.m. of manganese.

The lowest manganese content of healthy oats was found in those plants growing in jars B 7–9 and averaged 14·8 p.p.m. The total growth in these jars, although quite healthy, was not as great as in jars B 10–12, and it would thus appear that the dry weight of the crop produced was limited by the amount of manganese supplied in the nutrient solution. Therefore it seems likely that the proportion of manganese found in the dry matter of these plants is approximately the minimum quantity to be expected in healthy oats at this stage of development.

When the amount of manganese in the nutrient solution was 1 : 4,000,000 (1927) and 1 : 5,000,000 (1928) the amount of manganese in the dry matter averaged 21 p.p.m. In each season optimum growth was obtained in these jars. With nutrient solutions of greater manganese concentration larger amounts of manganese up to 83–96 p.p.m. were found in the dry matter, but in each case the growth was practically the same as at the lower concentration. Thus at least five to seven times as much manganese as appears to be necessary for growth can be present without affecting the plant in either way.

A number of samples of Algerian oats from different localities were examined to determine how their manganese content compared with that of the plants grown in water-cultures. Table V shows the localities selected and the amount of manganese found.

Table V.

Showing manganese content of Algerian oats at flowering stage.

Locality	Mn p.p.m.	Remarks
(1) Penola	10·2	Diseased
(2) Mt Gambier ...	10·4	,,
(3) Mil Lel	10·3	,,
(4) Mil Lel	14·3	Diseased but recovered somewhat
(5) Wasleys	20·4	Healthy
(6) Bundaleer Valley ...	24·7	,,
(7) Riverton	35·7	,,
(8) Belalie North ...	47·5	,,
(9) Waite Institute ...	82·0	,,

The first three samples represent oats, all of which showed marked symptoms of manganese deficiency. Sample 4 was from a self-sown crop of Algerian oats across the road from sample 3. In its earlier stages this self-sown crop had exhibited symptoms of the disease, but with the approach of spring had recovered somewhat and produced some grain. Samples 5–9 represent oats from localities not subject to the manganese deficiency disease. From the analyses it will be seen that there is the same range of variation in manganese content in the last five samples as in the samples of healthy oats from the water-cultures.

The amount of manganese in sample 4 was almost identical with the minimum amount found in the water-culture plants that were making healthy growth, and is further evidence that this amount (about 14 p.p.m.) is the minimum quantity to be expected in normal Algerian oats at the flowering stage.

D. Experimental work relating to factors possibly influencing the appearance of manganese deficiency in oats in the field.

The following series of water-cultures was designed to test whether excess of calcium ions or the presence of traces of organic substances, as were claimed by Arrhenius (1924) and by Hudig (1923) respectively to influence the appearance of Grey Speck disease of oats in the field (which was shown by us (1928) to be identical with manganese deficiency), would also have an influence on the availability of manganese in water-cultures.

(a) *The effect of excess of calcium ions in water-culture.*

Solutions with excess of calcium ions in different proportions were prepared in the following way:

The sodium chloride of the standard solution was reduced to 0·1 gm. per litre, and calcium chloride was added to raise the proportion of calcium ions to the four ions Ca, Mg, Na, K, to the desired degree. In one series the proportion of calcium ions was raised to 80 per cent., the solution being used diluted to quarter strength. In another series the proportion of calcium ions was raised to 50 per cent., the solution being used both undiluted and diluted to two-thirds strength. Three similar series were prepared in which calcium nitrate was added instead of calcium chloride.

Three jars of each solution were prepared, to two of which manganese 1 : 5,000,000 was added, the third being left manganese-free.

All plants in the manganese-free jars showed symptoms of manganese deficiency within four to six weeks as usual, but at no time did symptoms of manganese deficiency appear on any of the plants in jars containing manganese, however high the proportion of calcium ions (Plate XXIII, fig. 2). Excess of calcium ions therefore does not render manganese unavailable if this is present in the soluble and highly ionized form of sulphate (or probably of any soluble inorganic manganese salt).

(b) *The effect of excess of nitrate ions in water-culture.*

It has been noted in many field experiments on the Grey Speck disease of oats that nitrate of soda tends to increase the severity of the disease. Nitrate of soda was added to six jars of manganese-free culture solution (in which the sodium chloride was reduced to 0·2 gm. per litre) to the extent of 0·15 per cent. in three jars and 0·3 per cent. in three jars. To two jars of each series Mn 1 : 5,000,000 was added.

Again the plants in the manganese-free jars developed typical symptoms of manganese deficiency, whereas all plants in jars containing manganese grew normally, notwithstanding the presence of a considerable quantity of nitrate of soda.

(c) *The effect of the presence of organic compounds.*

The following water-culture series was arranged:

G 1–3	+"humus" from Mn-deficient soil	2 Mn 1 : 5,000,000 jars,	1 Mn-free jar
G 4–6	+"humus" from sugar	,,	,,
G 7–9	+sucrose, 0·05 %	,,	,,
G 10–12	+dextrose, 0·05 %	,,	,,
G 13–15	+starch, 0·05 %	,,	,,
G 16–18	+cellulose, 0·05 %	,,	,,
G 19–21	free of organic matter	,,	,,

As was to be expected, there was a growth of fungi over the surface of the solutions containing sugars, but this did not interfere with the determination of symptoms of manganese deficiency. Typical symptoms of manganese deficiency appeared in all the plants grown in manganese-free solutions, but were not at any time observed on plants in solutions containing manganese sulphate, whichever of the above organic substances was present in the nutrient solution.

It is thus evident that the factors, (1) excess of calcium ions, (2) excess of nitrate ions, or (3) the presence of traces of organic compounds in the nutrient solution, are not in themselves the cause of manganese deficiency in oats. It remains possible, however, that they may act in combination

with some other factor or factors as yet undetermined in rendering manganese unavailable in certain soils.

(d) *Chemical determinations of manganese in plants from manganese deficient soils as compared with that in plants from normal soils.*

To strengthen the argument advanced in our previous paper (1928), derived from soil percolation tests, that manganese is less available in the soils on which oats suffer from manganese deficiency, the following series of analyses was done on various plants and weeds, all of which, with the exception of oats and barley, appeared just as healthy and vigorous on the manganese deficient soil as on the normal soil.

The normal soil from which the plants were collected was the red clay loam at the Waite Institute, on which no deficiency disease appears. Corresponding samples of plants were always selected at the same stage of growth.

Table VI.

Showing the manganese content of plants from normal and manganese deficient soils.

Results expressed as parts of manganese per million parts of dry matter.

Plant	Mn content when growing on a normal soil. (Waite Institute) p.p.m.	Mn content when growing on Mn-deficient soil p.p.m.	Locality from which Mn-deficient sample obtained
Algerian oats, 11–15 weeks after seeding	73·1*	7·1†	Penola
		18·5‡	Mt Gambier
Algerian oats, nearly ripe	76·6	10·2	Penola
		13·9§	Mt Gambier
Lachlan oats, flowering	71·4	13·2§	Mt Gambier
Cryptostemma, Calendulaceum (Capeweed)	72·1	30·9	,,
Poa pratensis	37·0	30·3	,,
Barley	26·4‖	7·65†	Corney Point
Bromus maximus	57·1	11·6	,,
Sonchus oleraceus	74·1	16·8	,,
Lucerne	—	17·4	,,
Lolium temulentum	—	7·5	,,
Perennial rye grass	—	11·5	Mt Gambier

* Average of 6 samples.
† Average of 4 samples (diseased).
‡ Average of 5 samples (diseased).
§ Average of 2 samples (diseased).
‖ Grown in pots (1926).

From the table it will be seen that the manganese content of all the plants examined from the Waite Institute was much greater than that of the same species growing at any of the other three places. The difference was least in the samples of *Poa pratensis*, the Waite Institute

sample being only 21 per cent. richer in manganese than the sample from Mt Gambier. The greatest difference was shown in the oats from Penola and the Waite Institute, the latter samples containing on the average ten times as much manganese as the former. In all other cases the samples taken from the Waite Institute contained $2\frac{1}{2}$ to $7\frac{1}{2}$ times as much manganese as the corresponding sample from a manganese deficient soil.

These differences are all of a greater order than the normal variation from plant to plant as found in the examination of single oat plants, and, as composite samples were always taken so as to represent the average for the particular locality, the variations found must be taken to represent variations in the availability of manganese in the different soils.

III. DISCUSSION.

The two most important points brought out by the above work are: (1) that manganese is an essential element for the growth of all the plants tested, being essential from an early seedling stage, and (2) that different plants require different amounts of manganese to enable them to complete their development. The latter is the explanation of the fact that certain types of soil, which do not possess sufficient available manganese for the growth of oats, nevertheless support an apparently normal growth of pasture plants and weeds. At the same time such pasture plants and weeds have a considerably lower manganese content than similar plants from normal soils. It seems possible that this fact may later be found to have some connection with certain animal diseases which occur on these manganese deficient soils in South Australia.

The question of what factor or factors is responsible for the poor availability of manganese in these soils is still unsolved. Oats have been found to suffer from manganese deficiency on three widely differing types of soil in South Australia. These are: (1) a rich, brown, volcanic ash soil from round Mounts Gambier and Schank; (2) a black clay-humus reclaimed swamp soil from Penola; and (3) a light calcareous soil from the foot of Yorke's Peninsula, Eyre's Peninsula and Kangaroo Island.

That the poor availability of manganese on these soils is connected with the soil reaction becomes clear from the fact that the patches on which oats are most badly diseased are always more alkaline in reaction, and from the fact that liming increases the severity of the disease. There are many other soils as alkaline as these, however, and possessing no more manganese, on which the trouble does not appear.

Moreover, under certain conditions the soils in which manganese is normally unavailable in sufficient quantity for the growth of oats, may

so change, without manurial treatment, that abundant manganese becomes available.

An illustration of this is furnished by a series of pot experiments which was designed to investigate the effect of soil reaction on the manganese deficiency disease of oats. The series consisted of sixteen (glazed) pots of soil from a field at Mt Gambier on which oats suffered from manganese deficiency. The soil for these pots was treated with hydrochloric acid or calcium carbonate in such a way as to produce a range of hydrogen ion concentration from *p*H 5·5 to *p*H 8·0 in eight steps, there being two pots of each reaction.

Table VII.

Showing an unexpected result in a series on the influence of soil reaction on manganese deficiency disease of oats.

Pot no.	Treatment	*p*H expected	*p*H found (14. vi. 28)	Remarks	Mn in crop (3. x. 28) p.p.m.
1 and 2	187·6 ml. HCl	5·5	5·6	Healthy	290
3 and 4	141·5 „	5·9	6·0	„	90·4
5 and 6	92·3 „	6·4	6·9	„	14·4
7 and 8	67·7 „	6·7	7·0	„	8·8
9 and 10	39·0 „	7·1	7·4	Diseased	11·4
11 and 12	20·5 „	7·4	7·5	„	
13 and 14	Untreated	7·7	8·0	Healthy	115·8
15 and 16	30·75 g. $CaCO_3$	8·0	8·2	„	134·5

From experience, as detailed in our previous paper (1928), it was expected that the manganese deficiency disease would appear on the oats in all pots with a reaction more alkaline than about *p*H 7·0. It was expected that the trouble would be more severe in the pots treated with calcium carbonate than in the untreated pots; that it would be slightly less severe in the pots treated with such small amounts of acid as to leave the reaction still on the alkaline side; and that it would not appear at all in the pots made more acid than about *p*H 7·0.

As was anticipated (see Table VII) the disease did not appear in the pots more acid than *p*H 7·0; it did appear in the pots of reaction *p*H 7·4–7·5, which had been treated with small amounts of acid, but it did not appear, where it had been expected that it would, in the untreated pots (*p*H 8·0), or in those treated with calcium carbonate (*p*H 8·2). The plants were harvested after fifteen weeks' growth and the manganese present in the tops determined. Corresponding with the deficiency symptoms, it was found that the plants from the slightly acid-treated

pots which showed the disease had little manganese, whereas the plants from the untreated and the calcium carbonate treated pots, which had remained healthy, had much more manganese.

This unexpected result can only be explained by a difference in the method of filling the untreated and calcium carbonate treated pots from the acid treated ones. The soil for the whole series had been air-dried and sieved, and the various amounts of acid required, diluted in a definite volume of water, had been thoroughly mixed into the soil by hand stirring so that an evenly moist sample was obtained before filling into the pots. The measured amount of calcium carbonate was thoroughly mixed with the air-dry soil in a revolving box, and this, and also the soil for the untreated pots, was filled into the pots dry. When these pots were watered the soil must have become packed very tightly as it swelled, and this appears to have greatly influenced the availability of the manganese.

In this connection the experience of Godden and Grimmett (1928) that the manganese content of oats in undrained pots was about six times that of oats in drained pots is interesting.

It has also been noted in field experiments on manganese deficient soils in South Australia that rolling is beneficial for the growth of oats, and if the crop has been sown with the aid of a tractor the improved growth in the track of the tractor wheels is most noticeable.

These examples would suggest that the oxidation reduction potential of the soil is well worth investigating in further work on the factors influencing availability of manganese in soils.

A further point worthy of attention arises from a consideration of the *symptoms* of manganese deficiency. The arrest of shoot growth, with formation of small chlorotic leaves at the tips, and slightly lower down the characteristic green network of veins with yellowed mesophyll tissue between, recall at once the symptoms of several well-known diseases the cause of which has long been obscure. Pecan rosette, mottle-leaf of citrus, and little-leaf or yellows of walnut are perhaps the three most important diseases thus called to mind. No tests were done with these plants in the present investigation, and so no more detailed discussion of the symptoms can be undertaken here. But besides the apparent similarity between the symptoms of these diseases and those of certain other plants known to be suffering from manganese deficiency, there is also some similarity in the mode of occurrence. There is evidence that both pecan rosette and mottle-leaf of citrus are made worse by applica-

tions of lime, and that walnut yellows is increased on plots heavily treated with nitrate of soda for some years. Certain relations to the amount of humus present in the soil also correspond with what has been found for some naturally occurring manganese deficiency diseases. At all events, the similarities which exist suggest that it would be fully worth while to determine by experiment whether the pecan rosette, the mottle-leaf of citrus and walnut yellows are not possibly manganese deficiency diseases.

The writers desire to acknowledge their indebtedness to Prof. J. A. Prescott for helpful criticism in the preparation of this manuscript.

IV. SUMMARY.

1. The conclusions of certain previous investigators that manganese is an essential element for plant growth have been confirmed by means of water-cultures.

2. Manganese becomes necessary to plants at an early seedling stage, and remains necessary until a late stage in growth.

3. The symptoms of manganese deficiency are essentially an arrest of development followed by death of the undeveloped tissues at the growing points, and the use of the word chlorosis as the primary symptom is misleading. In many cases a special type of chlorosis of the upper parts of the plant does occur before death of the tissues.

4. Rye developed to maturity in water-cultures on an amount of manganese which did not allow the complete development of oats.

5. A concentration of manganese of one part in fifty million parts of nutrient solution allowed complete development of rye, and would probably be sufficient for oats also if a method of continuous solution change was arranged.

6. Evidence is given that the minimum quantity of manganese which will allow healthy growth of Algerian oats is about 14 parts per million of the dry matter at the flowering stage.

7. On certain soils oats are unable to absorb this minimum quantity, and they then suffer from the Grey Speck disease, a true manganese deficiency disease which can be cured by application of a soluble manganese salt.

8. On soils with abundant available manganese considerably more manganese is absorbed by oats than is required for normal development. The amount absorbed varies greatly, and in all probability depends to some extent on soil reaction and soil aeration.

9. Analyses are given showing that a number of plants, when

growing on a red clay loam soil on which no deficiency disease occurs, have a much higher manganese content than similar plants taken from soils on which oats suffer from manganese deficiency.

10. The variation in ash and manganese content during the growth of the oat plant is shown in Table II.

11. Such factors as the presence of organic matter, or excess of calcium ions, which have at various times been claimed as causes of the Grey Speck disease of oats have been shown to play no part in the appearance of this trouble in water-cultures where a soluble manganese salt is present. These factors cannot themselves render manganese unavailable when it is present in a soluble form. This does not exclude the possibility that they may later be found to have some effect, in conjunction with other factors as yet undetermined, in rendering manganese unavailable in certain soils.

12. None of ten different rarer elements tried, nor the combination zinc, copper, boron and aluminium, was able to replace manganese in the growth of oats.

V. APPENDICES.

I. *Water-culture methods.*

The seed of cereals was germinated on waxed mosquito netting fitted over a jar of distilled water. The seed of smaller plants—clovers, grasses, tomato, etc.—was germinated in a pure sand which had been boiled with hydrochloric acid and well washed with distilled water. When the cotyledons had expanded the seedlings were washed out from the sand and transferred to the holes in waxed mosquito netting over a beaker of manganese-free culture solution until they were sufficiently big to set out in the culture jars.

The culture vessels used were rectangular museum jars of capacity 3·2 litres, covered on the outside with black paper and coated on the inside with a thin layer of paraffin wax. When solutions were changed fresh jars were used if the paraffin wax showed signs of flaking off. The rectangular shape of jar allowed plants to be readily lifted out for change to another jar, inspection of roots, etc.

The seedlings were supported by means of a little paraffined cotton wool in holes in rectangular wooden covers which had been treated with raw linseed oil and later dipped in melted paraffin wax. There were six holes per cover for seedlings, and one through which a bent aerating tube was fixed, this also being paraffined where it dipped into the solution.

Seedlings were set out in the culture jars at as small a stage as they could be conveniently handled. From one to six seedlings were used per jar, according to the experiment; in the majority of cases six was the number used.

The Rothamsted culture solution, with ferric citrate substituted for ferric chloride, was used throughout the work except for certain leguminous plants for which Brenchley's modified solution of reaction *p*H 6·2 was used.

Precautions for the exclusion of manganese. Ordinary distilled water from a Stokes

still was used in the making of all culture solutions. As described above, the insides of all culture jars, and the glass aerating tubes, were paraffined, so that solutions did not come in contact with glass after they were prepared. In an experiment in which oats were grown in a manganese-free culture solution in both paraffined and unparaffined jars, it was found that the plants in the unparaffined jars grew larger and lived longer than those in the paraffined jars, which were completely dead after some three months' growth. The controls with manganese 1 : 5,000,000 were equally vigorous in both, and were only discarded when three feet high. It seems probable that a still greater difference between plants grown in unparaffined and paraffined jars, due to absorption of manganese from the glass in the former, would have been evident if plants with a smaller manganese requirement than oats had been used.

Analytical reagents were used in the making of culture solutions, and these were all carefully tested and shown to be free from manganese by the periodate test. It was however found impossible to purchase an iron salt free from manganese. The presence of manganese in the purest reagent iron salts obtainable was shown both by the periodate test, and by water-cultures with plants. In an experiment in which oats were grown in a "manganese-free" culture solution prepared with iron salts guaranteed free of manganese as purchased, the plants grew much further than those in solutions really free from manganese, made with the specially prepared manganese-free iron citrate described below. Again it seems probable that if plants with a smaller manganese requirement than oats had been used the difference would have been considerably greater, and possibly no symptoms of manganese deficiency would have been evident at all with some plants in solutions prepared with ordinary A.R. iron salts.

Preparation of manganese-free ferric citrate. In order to obtain larger quantities of iron-free from manganese an electrolytic deposition method was used instead of the modified basic acetate separation noted in our previous paper (Samuel and Piper, 1928). The iron was deposited from a hot 14 per cent. solution of the purest obtainable ferrous sulphate on to a platinum cathode. The anode was a bar of iron separated from the cathode by a porous cell. This porous cell served to retain any manganese precipitated as the dioxide at the anode. The electrolyte was replaced every two or three hours by a fresh solution of ferrous sulphate. At the same time the iron, deposited on the cathode, was removed by solution in pure concentrated hydrochloric acid.

When sufficient ferrous chloride had been thus prepared it was recrystallised from the hot hydrochloric acid solution. It was then oxidised to ferric chloride by boiling with a small excess of pure nitric acid, this excess being removed by evaporation with a little hydrochloric acid on a water bath.

The ferric chloride so obtained was diluted to a definite volume and iron determined in an aliquot portion. In another portion, corresponding to 4–10 gm. of iron, the absence of manganese was proved using the periodate test.

The remainder of the ferric chloride solution was evaporated in a silica basin after the addition of sufficient pure citric acid to secure about two-thirds conversion to ferric citrate. This evaporation removed most of the free hydrochloric acid. The mixture of ferric chloride and citrate, left as a pasty mass, was dissolved in warm water, filtered, made to a definite volume, and kept in a paraffined flask. It was used in this form in the preparation of the nutrient solutions.

II. *Method for the chemical determination of manganese in plants.*

Composite samples have been taken by cutting plants as close to the ground as possible, precautions being observed to prevent contamination with soil. After air-drying the sample was hand-picked to eliminate dust and traces of soil. It was then oven-dried and ground in a Wiley Mill, using a 1 mm. screen. Manganese has been determined throughout by a colorimetric method after ashing and oxidation to permanganate. Owing to its superiority potassium periodate has been used to bring about this oxidation (Willard and Greathouse, 1917). A Dubosq type colorimeter with 100 mm. tubes has been used for making all colour comparisons. When insoluble matter, such as calcium sulphate, renders the colour solution turbid, it can easily be removed by centrifuging for 1–2 minutes immediately before making the colour comparison.

The details of the method are as follows:

5–25 gm., according to the amount of manganese present, of the oven-dried sample were ashed as completely as possible in a silica basin. The ashing was started over a small burner and finished in an electric muffle at a dull red heat.

The ash was treated with 25 ml. of dilute hydrochloric acid (1 + 1) and digested for 15 minutes under a clock glass on a water bath. It was then evaporated to dryness and left on the bath for about an hour to render silica insoluble. The residue was taken up with 25 ml. of hot water and 5 ml. of conc. nitric acid, filtered, and washed once or twice with hot water and two lots of warm dilute nitric acid. The washing was finished with hot water alone.

The filtrate was transferred to a silica basin and evaporated to dryness on a water bath. 25–30 ml. of dilute sulphuric acid (1 + 1) and 5 ml. of nitric acid were added and the evaporation continued on the water bath and finally on a hot plate or sand bath until fumes of sulphuric acid had been produced for about a minute.

When cold the contents of the basin were diluted with 30 ml. of water and 1–1·5 ml. of phosphoric acid. 0·3–0·5 gm. of potassium periodate was added and the solution in the basin boiled after the production of the permanganate colour. If the colour was sufficiently intense a further 25–30 ml. of water were added during boiling. The solution was then transferred to a suitable volumetric flask (50 ml., 60 ml. or 100 ml.) and diluted nearly to the mark. It was then placed in a boiling water bath for twenty minutes together with other flasks containing quantities of a standard manganous sulphate solution (from $KMnO_4$), sulphuric and phosphoric acids, and the potassium periodate. Useful standard colour solutions ranged from 0·07 mg. to 1·0 mg. manganese per 50 ml.

When cold, and after diluting to the graduation mark, the colour of the permanganate is matched against one of the above solutions of known strength.

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EXPLANATION OF PLATES XXII—XXIV

PLATE XXII.

(The scale is given by the water-culture jars, which are $9'' \times 6''$.)

Fig. 1. Peas (Brunswick White), 10 weeks
Fig. 2. Broad beans, 15 weeks
Fig. 3. *Trifolium subterraneum**, 16 weeks
Fig. 4. *Phalaris bulbosa*, 10 weeks

} On left, jar containing nutrient solution plus manganese 1 : 1,000,000 (as sulphate); on right, jar containing nutrient solution free from manganese.

* Manganese 1 : 1,000,000 was found to be toxic to the clovers, and after six weeks' growth the concentration of this element was reduced to 1 : 5,000,000, after which the plants grew forward in a healthy manner.

PLATE XXIII.

(The scale is given by the water-culture jars, which are $9'' \times 6''$.)

Fig. 1. Wheat (Late Gluyas) and barley (Cape), both 20 weeks old, in nutrient solution plus manganese 1 : 1,000,000, showing stage to which cereals were grown before harvesting.

Fig. 2. Algerian oats, 18 weeks old, in solutions containing excess of calcium nitrate to raise the proportion of calcium ions to the ions Ca, Mg, Na, K to 50 per cent. On left, jar containing this nutrient solution plus manganese 1 : 5,000,000; on right, jar containing the nutrient solution free from manganese.

Fig. 3. Barley (Cape), 16 weeks old. On left, jar containing nutrient solution plus manganese 1 : 1,000,000; on right, jar containing nutrient solution free from manganese.

Fig. 4. *Bromus unioloides*, 10 weeks old. On left, jar containing nutrient solution plus manganese 1 : 1,000,000; on right, jar containing nutrient solution free from manganese.

PLATE XXIV.

(Symptoms of manganese deficiency on oats.)

Fig. 1. Oat seedling grown on a soil deficient in available manganese, showing the typical symptoms of manganese deficiency in the early stages. Note the first leaf healthy, second and third leaves collapsed below the centre of the blade.

Fig. 2. Leaves from a plant of Algerian oats grown on a soil deficient in available manganese. Note the dead spots and streaks along the margins, characteristic of manganese deficiency on older plants. The presence or absence of a reddish margin to some of the spots is a variable feature, and is probably largely due to cold.

Fig. 3. Leaves from plants of Algerian oats grown in water-cultures free from manganese. Note the dead spots and streaks along the margins and correspondence with field symptoms of manganese deficiency as in Fig. 2.

(*Received May 10th*, 1929.)

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

SAMUEL & PIPER.—Manganese as an Essential Element for Plant Growth (pp. 493–524.)

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

SAMUEL & PIPER.—Manganese as an Essential Element for Plant Growth (pp. 493–524).

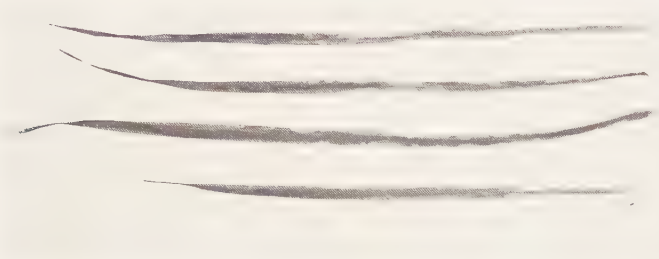

Fig. 3.

Fig. 2.

Fig. 1.

SAMUEL & PIPER.—Manganese as an Essential Element for Plant Growth (pp. 493–524).

A MOSAIC VIRUS OF GRASSES, NOT VIRULENT TO SUGAR CANE

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The leaf-mottling, typical of a mosaic disease, is commonly to be found in a number of grass species when growing in the vicinity of mosaic-diseased sugar cane (*Saccharum officinarum* L.). This condition has generally been assumed to be due to the cross-transmission of a single virus between the several hosts. There is experimental support for this view. Brandes (2), Kunkel (4), Hadden (3) and others successfully produced the mosaic pattern in healthy plants of sugar cane, maize (*Zea Mays* L.), *Sorghum* spp. and other grasses by experimental transmission of a virus from sugar cane and others of these hosts. In consequence it has generally been supposed that all typically mosaic-diseased grasses were affected by a common virus and were reservoirs whence the virus might be carried to sugar cane.

In the course of a search for wild host-plants of the sugar cane mosaic virus in South Africa, I encountered evidence which threw doubt upon this conjecture. Whereas I had no reason to suppose that the mosaic-diseased grasses in the sugar cane area of Natal were not carrying the sugar cane virus—and experiments later showed that the virus might be transmitted from certain of them to sugar cane—yet I found a mosaic widespread in maize and *Sorghum* spp. in the Transvaal where no mosaic-diseased sugar cane was known to exist.

This Transvaal virus produced signs in maize and Sorghums indistinguishable from that caused by the sugar cane virus in these hosts. Nevertheless my experiments led me to believe that this virus is incapable of producing any visible effect upon sugar cane. I have stated this conclusion in a previous brief report (7). I now amplify the details of the evidence upon which it is based.

This work was carried out at the Natal Herbarium, Durban, under the direction of Dr I. B. Pole Evans. I acknowledge the assistance of J. S. Mackay and R. F. W. Nichols.

Field observations.

The Transvaal mosaic was first observed in 1924 in plants of the wild grass species, *Sorghum arundinaceum* Stapf[1], which had been collected in the neighbourhood of the Groenkloof Experiment Station, near Pretoria, and had been planted in a plot in this station. In subsequent years the disease was evident in this plot and in a second plot arising from roots transplanted to the Prinshof Experiment Station, Pretoria. Diseased plants of this species were not found elsewhere in the Transvaal, but no considerable search for them was made. A mosaic was, however, found in maize and cultivated Sorghums, both of the grain and sweet types, on many farms in the Rustenburg and Waterberg districts of the Transvaal. In these areas a number of small plots of sugar cane, of varieties known to be susceptible to mosaic, were at this time all found to be free from mosaic disease[2].

Although I have not demonstrated the identity of the viruses causing the mosaics in *Sorghum arundinaceum* at Pretoria and in maize and cultivated Sorghums in the neighbouring Rustenburg and Waterberg regions, yet I have no reason to doubt their identity. My experiments in cross-transmission were, however, performed with *Sorghum arundinaceum* only.

In Natal meanwhile a mosaic of sugar cane and a number of grasses was prevalent and was spreading freely to susceptible varieties of sugar cane. I have recorded my observations upon this mosaic in an earlier paper (5). For reasons there stated I believe this disease to have been introduced into Natal within recent years in cane sets imported from the New World, and therefore to be identical with the sugar cane mosaic known in most cane-growing countries.

Signs of the disease.

The signs of the common sugar cane mosaic in sugar cane, maize and Sorghums are well known and have been frequently described (*e.g.* (1), (4), (5)). To these the signs of the Transvaal mosaic in the several hosts have closely conformed, and I have found no visible character for separating it from the common mosaic, as shown by affected plants

[1] *Sorghum arundinaceum* Stapf. Determined by Miss Stent, National Herbarium, Pretoria. A weak tufted perennial under South African conditions. Described by Stapf as an annual in *The Flora of Tropical Africa*, IX, Pt. I, p. 114, and originally included by him with *S. verticilliflorum* in *Andropogon halepensis* var. *effusus* (*Flor. Cap.* VII, 346).

[2] A recent unconfirmed report records mosaic in a plot of cane at Warmbaths, Transvaal.

in Natal. The tendency, observed by Brandes (1), for the mosaic pattern to become suppressed as the leaves age was evident in maize and Sorghums when affected with either virus. Indeed in affected *Sorghum arundinaceum* plants the mosaic pattern was often barely visible even on the youngest leaves. On the other hand in the field in the Waterberg maize frequently exhibited an unusually pronounced contrast between the light and dark green areas of the mosaic-affected leaf. This deviation from the usual type was not shown by maize plants of the Hickory King variety when experimentally infected with the Transvaal virus taken from *Sorghum arundinaceum*. These plants were indistinguishable from those infected with the Natal virus. While this evidence might be held to indicate that the Waterberg maize virus was distinct from the Pretoria Sorghum virus, it is quite insufficient to prove this point. I consider it to be more probable that climatic conditions or varietal differences account for the pronounced manifestations observed in the Waterberg.

Experimental methods and results.

Cage experiments. Experiments were carried out in an insect-proof greenhouse to determine whether *Aphis maidis* Fitch, the known vector of sugar cane mosaic, could transmit the Transvaal mosaic. Groups of aphides were caged upon single leaves of the experimental plants, by means of the glass tubes described elsewhere (6). Mosaic was thus successfully transmitted to maize by aphides collected on diseased maize,

Table I.

Transvaal mosaic.

Tests for transmission to maize, by *Aphis maidis*. Glass-tube leaf-cage method in greenhouse, 6–12 aphides to each plant. Mosaic appeared in 8–30 days.

Date	Source of infection	Tests on maize		Controls		Details
		No.	Diseased	No.	Diseased	
11. ii. 25–23. iv. 25	Mosaic maize	12	10	12	Nil	Aphides tested immediately after collection upon diseased plants in the field in the Transvaal
	Mosaic cultd. Sorghum	8	7	8	Nil	
	Mosaic, *Sorghum arundinaceum**	15	4	15	Nil	
26. vi. 25–20. x. 25	Mosaic, *Sorghum arundinaceum*	30	12	30	Nil	Non-infective aphides from cultures on healthy plants fed for a time upon the diseased Sorghum plants

* Aphides taken from diseased plants in the Groenkloof and Prinshof experiment stations, Pretoria.

cultivated Sorghums and *Sorghum arundinaceum* (Table I). Aphides, which were previously non-infective but were fed for a period upon a diseased *Sorghum arundinaceum* plant, were also able to transfer the mosaic to maize.

For experiments in transmission to sugar cane, large cages were constructed at Durban, Natal, covering an area of 8 feet square, with glass roofs and wire-gauze sides. The experimental cane plants were raised from sets in tins within the cages, together usually with a number of maize seedlings. Diseased plants, of the kind to be used as the source of infection, were placed between the experimental plants. When all plants were through the ground and growing rapidly, large numbers of *Aphis maidis* were distributed upon the diseased plants. After a period of feeding and development on the diseased plants, the aphides moved naturally to the healthy ones, upon which they were usually to be observed feeding. Aphid-distribution was repeated several times. The experimental plants were retained under observation in the cages for about three to five months.

An adjacent cage was occupied by control plants, similarly treated except that there were no diseased plants and no aphides were distributed. The most important function of these controls was to ensure that no cane sets were already carrying the mosaic virus at the time of planting; consequently at least one control set was taken, usually from the top, from each whole cane, which was cut into the sets which provided the experimental plants. During these experiments no control plant developed mosaic.

Aphides for distribution in the whole series of experiments were reared upon healthy Sorghum seedlings. They were all the progeny of one original non-infective culture. At intervals samples of the aphides were removed from the culture cages and tested for infective power by caging on maize or cane. In five tests all of the plants remained free from mosaic disease[1].

Experiments in the transmission of the Natal virus were thus carried out, the sources of infection being sugar cane, *Sorghum arundinaceum* and *Setaria sulcata* Raddi, all diseased specimens collected in the field in the Natal cane area. No experiment failed to give some positive infections of sugar cane, although the infections occurred with that

[1] The tests were as follows: On 9. x. 25 groups of aphides tubed on 8 maize plants; on 23. iii. 26 caged with 30 maize plants; on 1. vii. 26 caged with 29 maize plants; on 17. xii. 26 caged with 41 cane plants; on 14. iv. 27 caged with 44 cane and 62 maize plants. All plants remained free from mosaic disease.

irregularity which is commonly experienced in experiments in mosaic transmissions (Table II).

Table II.

Natal mosaic.

Large cage experiments in transmission to sugar cane and maize. Cane varieties infected—Port Mackay, Rose and Striped Bamboo, Gingor, P.O.J. 213, D. 1135, Q. 813, 1900 seedling, Co. 210, Co. 213, and four varieties of uncertain relations.

Date	Source of infection	Cane plants exposed		Maize plants exposed		Control cane plants	
		No.	Diseased	No.	Diseased	No.	Diseased
1. vii. 26	Mosaic cane, variety P.O.J. 213	77	30	44	10	52	Nil
17. xii. 26	Mosaic cane, variety Rose Bamboo	15	6	—	—	37	Nil
14. v. 27	Mosaic cane, several varieties	52	43	—	—	80	Nil
9. i. 26	Mosaic *Setaria sulcata*	42	12	24	1	36	Nil
14. iv. 27	Mosaic *Setaria sulcata*	25	6	—	—	36	Nil
25. xii. 25	Mosaic *Sorghum arundinaceum*	72	12	48	48	30	Nil

Experiments designed to transmit the Transvaal mosaic to sugar cane were performed in a similar manner, employing the same strain of aphides. The source of infection was diseased *Sorghum arundinaceum* transplanted from Pretoria. In two experiments, the results of which are summarised in Table III, all of 100 cane plants, of varieties known to be susceptible to the Natal virus, remained free from all signs of mosaic. Meanwhile mosaic developed in each experiment in maize seedlings exposed, and in one in *Sorghum arundinaceum* seedlings exposed.

Table III.

Transvaal mosaic.

Large cage experiments in attempted transmission to sugar cane, maize and *Sorghum arundinaceum*. Cane varieties used: Rose Bamboo, Port Mackay, Black Innes, D. 1135, 1900 seedling, Q. 813. Maize and Sorghum seedlings raised in cage from seed.

Date	Source of infection	Cane plants exposed		Maize plants exposed		*Sorghum arundinaceum* plants exposed		Controls	Remarks
		No.	Diseased	No.	Diseased	No.	Diseased		
9. x. 25	Mosaic *Sorghum arundinaceum*	56	Nil	80	17	15	11	36 cane plants—healthy. 15 Sorghum plants—healthy	Aphides observed feeding on *all* cane plants
26. iii. 26	Mosaic *Sorghum arundinaceum*	44	Nil	15	11	—	—	42 cane plants—healthy	Aphides observed feeding on many cane plants

Field experiment. A field experiment with the Transvaal mosaic was carried out at the Prinshof Experiment Station, Pretoria. In December, 1925, a plot was planted with about 50 sets each of five cane varieties known to be susceptible to sugar cane mosaic (D. 1135, Port Mackay, Rose Bamboo, Striped Bamboo, Black Innes), and alongside was planted a plot of mosaic-diseased *Sorghum arundinaceum* taken from the Groenkloof Experiment Station. On the adjacent land also were sown plots of maize, grain Sorghum and sweet Sorghum. During the following months the last plots all developed mosaic in a large proportion of the plants. Nevertheless the cane remained free from mosaic-symptoms, and ratoons in the seasons 1926–7 and 1927–8, still alongside the ratoons from the diseased Sorghum roots, were similarly healthy. During each season many colonies of *Aphis maidis* were to be seen upon the diseased *Sorghum arundinaceum* plants.

Discussion.

In this paper I endeavour to prove that the Transvaal virus is not virulent to sugar cane, but the negative evidence required for a proof of this kind is generally not easily obtained. Any experiment, purporting to demonstrate the immunity of a plant species to a disease, must meet the charge that suitable conditions for infection have not been provided.

I believe that my evidence for the immunity of sugar cane to the Transvaal mosaic will withstand this charge. With a virus disease the most trustworthy proof of the immunity of a plant species is afforded by its healthy growth in a region where the disease is known to spread readily to susceptible species. The Pretoria experiment provided evidence of this kind. Some 250 cane plants survived uninfected through three seasons, although maize and Sorghums alongside became diseased. The conditions of this trial were particularly rigorous, for the adjacent diseased Sorghum was in each season colonised by *Aphis maidis*, individuals of which were able to infect maize in my experiments. It is hardly conceivable that the cane plants were not frequently subjected to the feeding of aphides carrying the virus derived from the diseased Sorghum.

The healthy condition of the cane in the farm plots seen in the Transvaal affords some confirmatory evidence for its immunity to the mosaic of this region. This evidence is, however, not worth much since the cane might have accidentally escaped infestation by virus-bearing aphides. Similarly the possible recent occurrence of mosaic in Transvaal cane weighs little against my hypothesis, since mosaic-infected cane sets could have been carried from Natal into the Transvaal.

It might be argued, however, that the conditions in the Transvaal, though favourable to the infection of maize and Sorghums, were yet in some way unfavourable to the infection of cane. Conceivably the Transvaal race of aphides was incapable of feeding on cane. For this reason experiments were carried out at Durban, observing a technique which maintained, as nearly as was possible in a region where the cane mosaic was endemic, the conditions holding in a field trial. Furthermore, this method was always successful in the transmission of the Natal mosaic to cane, including one experiment where the source of the Natal virus was *Sorghum arundinaceum*. Nevertheless in two separate experiments all of one hundred cane plants resisted infection by the Transvaal virus from *Sorghum arundinaceum*. It is certain that the majority of the cane plants were fed upon by aphides, of a strain capable of infecting cane with the Natal virus, and actually carrying a virus virulent to the adjacent maize and Sorghum.

I therefore conclude that the Transvaal virus is not virulent to sugar cane, or at least, that it is incapable of producing the signs of mosaic disease in sugar cane. This conclusion denotes some difference in the Transvaal virus from the Natal (or common sugar cane) virus. Already several virus diseases of grasses have been recognised, separable by their signs and sometimes by their insect-vectors; as, for example, sugar cane mosaic transmitted by *Aphis maidis* and streak transmitted by *Balclutha* (*Cicadulina*) *mbila* Naude. Here, however, there is a definite difference of kind. The difference in the mosaics which I have studied is one of degree, of virulence to a range of host-plants.

Summary.

This paper records studies of a mosaic disease observed in maize and *Sorghum* spp. in the Transvaal, South Africa. In these hosts the signs produced were not distinguishable from those produced by the common sugar cane mosaic virus. Leaf-cage experiments showed that *Aphis maidis* Fitch was capable of transmitting the virus to maize.

In the part of the Transvaal where the mosaic was found all sugar cane seen was free from mosaic. Sugar cane failed to contract the disease, both in a field experiment extending over three years in the Transvaal and in large cage experiments in Natal. The method employed in the Natal experiments was always successful in transmitting mosaic to sugar cane when the source of infection was either diseased cane or diseased grasses collected in the neighbourhood of diseased cane.

It is concluded that the Transvaal virus is not virulent to sugar cane and is therefore different from the common sugar cane mosaic virus.

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OBSERVATIONS DURING 1927–28 ON THE INCIDENCE OF "RUSTS" ON VARIOUS SELECTED WHEAT VARIETIES, WITH SPECIAL REFERENCE TO THE INTENSITY OF YELLOW RUST, *PUCCINIA GLUMARUM*, ERIKS. AND HENN.

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The following observations have been made on the Cambridge University Farm on wheat varieties grown for the purpose of testing their resistance to *Tilletia caries*. In each case the seed, prior to sowing, had been very heavily contaminated at approximately the same rate with bunt spores. The size of the plots, varied but in any one set of experiments was constant, and was never less than 18 feet of drill. One hundred leaves from each variety were examined in the laboratory for the presence of rust and recorded as having none, slight, moderate, or severe. A leaf was recorded as severely rusted if the whole or $\frac{3}{4}$ of its surface was completely yellow with pustules, moderate if $\frac{1}{4}$ to below $\frac{3}{4}$ rusted, slight if from a few scattered pustules or streaks to below $\frac{1}{4}$ rusted. The number of leaves showing a moderate attack was added to the number showing a severe attack, the total expressed as a percentage of the whole sample (100 leaves) was taken, in what follows, as the "Intensity of yellow rust attack." This system of estimation was the same as that described recently (1), but unless otherwise stated the leaves were taken at random from clean and bunted plants; in some cases, however, separate estimations were given of the rust intensity on clean and bunted tillers. In such a crude classification the main difficulty was in determining whether a leaf was or was not attacked, since with many varieties there were peculiar fleckings on the leaf, and, on these, pustules of yellow rust might or might not appear. In some cases these fleckings merged into each other until the leaf was almost completely yellow, when the yellowing must have had much the same effect on the plant as a moderately bad attack of yellow rust, since photosynthesis on those leaves could not take place. It appears that flecks of this description may be caused by the failure of the pathogen to establish itself on the host (2). No estimations were made of brown rust, *Puccinia triticina*, or black rust, *P. graminis*,

but some observations were recorded. It is difficult to tell if a variety markedly susceptible to yellow rust is susceptible or resistant to brown rust since the latter has little or no chance of attacking it. The percentage of bunt in these varieties was also recorded, and this was estimated at harvest by taking a total head count or by harvesting 1000 ears at random.

In addition to the varieties mentioned in the tables a number of other varieties were also tested for their resistance or susceptibility to rust and bunt. These have included sixteen Australian varieties, Felix, Maharajah, Sultan, Rajah, President, Sirdar, Bearded Gluyas, Late Gluyas, Gluyas, King's White, King's Early, King's Red, Waratah, American 8, Warden, Canberra (all *T. vulgare* Host.). With the exception of Warden none showed very marked resistance to *P. glumarum*. The yellow rust intensity of this variety on July 10th was 8 and the bunt percentage was 20. The nearest approach to this figure was Canberra with a rust intensity of 40 and a bunt percentage of 43. In this test the leaves were taken at random from clean and bunted tillers.

With these varieties it was interesting to note the rapid spread of yellow rust between June 28th and July 10th. Prior to June 28th a number of these varieties were free from rust, but after July 10th with the exception of these two, they had a rust intensity of 100.

Nineteen varieties resistant in America to strains of brown rust *P. triticina* were also tested, they included Warden, Hope, Chinese, Resaca, Malakoff, Hussar, Norka, Democrat, Mediterranean, Webster and Fultz (two selections). These varieties are all *T. vulgare* Host. Brown rust was not observed on any of these, but black rust was noted on Resaca, Hussar, Democrat, Mediterranean and Fultz. Yellow rust was observed on all the above varieties and the intensity was high. Warden on July 3rd had a rust intensity of 2, but this increased until July 23rd when the rust intensity was 90; it should, however, be stated that the bunt percentage was 80. Democrat and Mediterranean showed some resistance to yellow rust; in the latter case the rust intensity on June 17th was 0, but on July 25th it was 60, and the bunt percentage was 63.

In examining Table I it is of interest to note the following facts. With the exception of Marshal Foch, *T. vulgare* Host., there is a marked resistance to *P. glumarum*. This is especially noted in (B), *T. sphaerococcum* Perc., (D), *T. durum* Desf., American Club, *T. compactum* Host., Rivet, *T. turgidum* L. and (F), *T. monococcum* L. The resistance of *T. monococcum*, *T. durum* and *T. compactum* appears to be complete. The resistance of Red Fife, *T. vulgare* Host., (E), *T. polonicum* L., (C),

T. spelta L. and Persian Black, *T. persicum* Vav., is less marked. It is of interest to note that *T. monococcum* with 14 chromosomes shows complete resistance to the common wheat pathogens, but it will be seen that almost complete resistance to one pathogen *P. glumarum* is also shown by *T. compactum* and *T. sphaerococcum*, 42 chromosomes; and *T. durum* and *T. turgidum*, 28 chromosomes. In estimating the resistance of a variety to yellow rust it is an excellent criterion to note the reaction of the variety to *P. glumarum* when it is bunted. The writer considers that true and false resistance may be estimated in this way. For example, American Club when bunted showed *no rust at all on the leaves*, although it was interesting to observe that there was very slight rust on the bunted ears.

Dr E. F. Gaines, from whom the varieties on Table II were received, states *in litt.*: "These varieties have been bunt free at Pullman, Washington, in 1927. We find Hope and Khapli to be resistant to stem and leaf rust, and according to field observations they are probably resistant to stripe rust, *P. glumarum*." These wheats were tested in triplicate plots, but one set of results only is given, since the second and third sets confirm the first.

In Table III Iumello, Marquillo (Ma × Turkey 11 : 21 : 27), Marquillo 6887, and Marquillo (Ma × Turkey 11 : 21 : 22) appear to be markedly resistant to *P. glumarum*.

Kota is slightly more susceptible to yellow rust than Marquis, and the hybrid from these (Ceres) is as susceptible as Kota. Of the four *durum* varieties, Iumello and Pentad show some marked resistance to yellow rust, but Iumello is the more resistant to both bunt and rust.

The *diococcum* varieties, Vernal Emmer and Khapli, had a rust intensity on July 11th of 100; Khapli was literally yellow with rust pustules and suffered the worst attack of any of the varieties that were grown in 1928, the intensity being higher than that which occurs on White Odessa when it is grown in this country. The *durum* and *diococcum* races are in the "Emmer" group and have the 28 chromosome number, the remainder of the above wheats are *vulgare* and have 42 chromosomes.

English varieties.

Nineteen English varieties were tested for their resistance to yellow, brown and black rust and also to bunt. These varieties included Benefactoress, Little Joss, Marshal Foch, Rector, Wilhelmina, Harvester Red and White Wonder. Leaves were examined from clean and bunted tillers. Rivet and Little Joss showed marked resistance when clean, but when

Table I.

Showing the intensity of yellow rust on certain races of wheat.

Variety	Date of observation	% of yellow rust					% of bunted ears	Remarks
		None	Slight	Moderate	Severe	Moderate + severe		
(A) *Triticum diococcum* Schubl. ...	... 28. vi. 28	3	59	38	0	38	37	Rust. Intensity when bunted, 100
(B) *T. sphaerococcum* Perc. ...	... 15. vii. 28	No yellow rust observed. Yellow lesions on leaves					20	Black rust observed moderately bad. More yellow lesions on leaves when bunted
(C) *T. spelta* L.	... 28. vi. 28	23	43	32	2	34	2	Brown rust not observed
Red Fife, *T. vulgare* Host. ...	... 28. vi. 28	49	47	0	4	4	25	Bunted ears have more yellow rust. Brown rust observed. Rust intensity when bunted 50
(D) *T. durum* Desf.	... 28. vi. 28	100	2	0	0	0	0	Brown rust, occasional pustules. Yellow rust or bunted ears
Persian Black, *T. persicum* Vav.	... 28. vi. 28	Rust very slight but much yellowing. Rust pustules not active					25	Ergot observed. Brown rust slight to moderate. 31. vii. 28
American Club, *T. compactum* Host.	—	Rust not observed					57	Brown rust bad. Rust intensity of leaves when bunted 0. Yellow rust or bunted ears
Marshal Foch, *T. vulgare* Host.	... 28. vi. 28	6	67	27	0	27	37	Brown rust observed.
	10. vii. 28	0	0	84	16	100	—	Rust intensity when bunted 100
Rivet, *T. turgidum* L. ...	... 28. vi. 28	93	7	0	0	0	56	Rust intensity when
	10. vii. 28	82	18	0	0	0	—	bunted 18. Brown rust observed
(E) *T. polonicum* L.	... 28. vi. 28	7	65	28	0	28	12	Rust intensity when
	10. vii. 28	54	38	8	0	8	—	bunted 80. Brown rust not observed
(F) *T. monococcum* L.	—	0	0	0	0	0	0	No brown rust. No black rust observed

Table II.

Showing intensity of T. caries *and* P. glumarum *on certain American spring wheat varieties.*

Variety	C.I. no. Row no.	Date of observation	% of yellow rust					% of bunted ears	Remarks
			None	Slight	Moderate	Severe	Moderate + severe		
Hope, *Triticum vulgare* Host. ...	341	20. vi. 28	2	29	57	12	69	2	No black rust seen. Leaves too shrivelled to see if brown rust was present
		29. vi. 28	5	0	7	38	90	—	
		10. vii. 28	—	—	—	—	100	—	
Quality, *T. vulgare* Host. ...	325	20. vi. 28	89	6	5	0	5	4	No black rust. Brown rust observed. Yellow rust intensity when plants are bunted 50. Mildew noticeable
		29. vi. 28	43	6	1	0	2	—	
		10. vii. 28	60	40	0	0	0	—	
Alaska, *T. turgidum* L. ...	308	20. vi. 28	40	33	27	70	27	12	Yellow rust intensity when bunted 80. No black rust or brown rust
		11. vii. 28	—	50	50	—	50	—	
Khapli, *T. dicoccum* Schubl. ...	310	20. vi. 28	3	3	16	78	94	26	Yellow rust and shrivelled nature of leaves prevented observations for brown rust
		11. vii. 28	—	—	—	—	100	—	
Marquis × Turkey, *T. vulgare* Host.	—	20. vi. 28	3	57	35	5	40	31	No black rust observed. Yellow rust and shrivelled nature of leaves prevented observations for brown rust
		11. vii. 28	0	0	30	70	100	—	

Table III.

Showing the intensity of T. caries *and* P. glumarum *on certain wheat varieties resistant in America to biologic races of* P. graminis.

Variety	CI. no. Row no.	Date of observation	% of yellow rust					% of bunted ears	Remarks
			None	Slight	Moderate	Severe	Moderate + Severe		
Ceres, *Triticum vulgare* Host. ...	6900	19. vi. 28	29	47	25	9	34	31	*Ustilago tritici* observed.
		21. vii. 28	—	—	—	—	100	—	No black rust observed
Mindum, *T. durum* Desf. ...	5296	19. vi. 28	32	35	24	11	35	24	Yellow rust very bad on
		21. vii. 28	—	—	—	—	100	—	ears. No black rust
Iumello, *T. durum* Desf. ...	1736	19. vi. 28	94	6	—	—	0	6	Yellowing of foliage. No
		21. vii. 28	Yellow rust on ears						brown rust observed. No black rust observed
Pentad, *T. durum* Desf. ...	3322	19. vi. 28	62	26	12	0	12	80	Yellowing of foliage. No brown rust observed. No black rust observed
Webster, *T. vulgare* Host. ...	3780	19. vi. 28	8	30	44	18	62	73	No black rust observed
		29. vi. 28	0	11	21	18	78	—	
		3. vii. 28	0	5	9	36	90	—	
		11. vii. 28	—	—	—	—	100	—	
Marquis, *T. vulgare* Host. ...	3671	19. vi. 28	42	50	8	0	8	6	Mildew noticeable. No
		29. vi. 28	3	12	22	3	50	—	black rust. Rust in-
		11. vii. 28	10	30	35	25	60	—	tensity of bunted plants 80
Vernal Emmer, *T. diococcum* Schubl.	3686	19. vi. 28	68	15	12	5	17	23	Mildew noticeable. No
		29. vi. 28	11	12	18	9	54	—	black rust
		11. vii. 28	—	—	—	—	100	—	

Khapli, *T. diococcum* Schubl. ...	4013	11. vii. 28	—	—	—	—	100	—	Strongly susceptible to bunt. No black rust
Velvet Don, *T. durum* Desf. ...	1445	19. vi. 28	63	25	12	0	12	46	Rust intensity of bunted plants 100. No brown rust. No black rust
		29. vi. 28	0	18	22	10	64	—	
		11. vii. 28	0	30	70	0	70	—	
Kota, *T. vulgare* Host.	5878	19. vi. 28	96	4	0	0	0	69	Mildew noticeable. Rust intensity and yellowing of bunted plants 100. No black rust
		29. vi. 28	31	19	0	0	0	—	
		11. vii. 28	0	0	40	30	70	—	
Marquillo, *T. vulgare* Host. ...	11. 21. 27	19. vi. 28	Not observed					—	Mildew noticeable. Rust intensity of bunted plants 74. No black rust. No brown rust
		29. vi. 28	48	2	0	0	0	42	
		10. vii. 28	Yellowing						
Marquillo, *T. vulgare* Host. ...	6887	29. vi. 28	Occasional pustules of yellow rust					20	Ergot observed. No brown rust observed. No black rust
Progress, *T. vulgare* Host. ...	6902	19. vi. 28	36	62	2	0	2	82	No brown rust observed. No black rust
		29. vi. 28	25	25	0	0	0	—	
		11. vii. 28	0	0	60	40	100	—	
Reliance, *T. vulgare* Host. ...	7370	19. vi. 28	10	51	36	3	39	15	No black rust
		29. vi. 28	0	0	3	47	100	—	
		11. vii. 28	—	—	—	—	100	—	
Marquillo, *T. vulgare* Host. ...	11. 21. 22	19. vi. 28	—	—	—	—	0	20	No rust noted on ears. Brown rust observed very slight. Brown neck. No black rust
		10. vii. 28	48	52	0	0	0	—	
Hope, *T. vulgare* Host. ...	8118	19. vi. 28	23	53	24	0	24	2	No brown rust. No black rust
		29. vi. 28	6	0	24	20	88	—	
		11. vii. 28	—	—	—	—	100	—	

bunted were susceptible, Rivet being less susceptible when bunted than Little Joss. Brown and black rust was observed on each of the 19 varieties, black rust was diagnosed in the teleutospore stage and the observations on this rust were made on August 14th. Brown rust generally develops extensively in East Anglia in the second or third week of June, it occurs too late to do serious damage. Black rust, from our own observations, on the University Farm occurs later still; in 1928 it was observed at the end of July, and on August 14th the teleutospore stage was observed on all the English varieties tested, but no estimations of its intensity were made.

Discussion.

Sax (3) concludes that there is a relationship between the chromosome number of the wheat races and their resistance or susceptibility to pathogens. He considers that the races with the lower chromosome number are more resistant than races of a higher number. He states: "The percentage of infection (*i.e.* bunt) in *T. polonicum* was 8, in *T. diococcum* 10, in *T. durum* 29, in *T. turgidum* 33. In the vulgare group *T. spelta* was comparatively resistant with only 10 per cent. infection, but the percentage of bunt found in *T. vulgare* was 70 and in *T. compactum* it was 64 per cent. In general, the species and varieties of wheat which are resistant to rust are resistant to bunt and *vice versa*. It is probable, then, that varieties found to be resistant to rust in the middle west would be valuable disease-resistant varieties for the Pacific coast where bunt is the most important cereal disease. Likewise the valuable results in breeding bunt-resistant wheats on the Pacific coast could be utilised in regions where rust is prevalent.... With an increase in chromosome numbers, 14–28–42, there is an increase in variability and adaptability, and increased susceptibility to rust, mildew and bunt, a better quality of gluten in the grain, and the economic value is greater. ...Rust and bunt resistance apparently depend on the same factors, so that results in breeding wheats resistant to rust can be applied to bunt and *vice versa*...."

From our own observations we would not accept these broad generalisations, for although it seemed clear that *T. monococcum* with its 14 chromosomes showed complete resistance to the common wheat pathogens, the 28 chromosome group when compared with the 42 group varied considerably in the relative resistance to disease organisms.

The races of wheat quoted by Sax were not contaminated with their own bunt, and the writer has shown (4) *that when this is done resistant varieties*

become susceptible. The writer considers that there is no firm foundation for believing that the species and varieties of wheat which are resistant to rust are resistant to bunt and *vice versa.* For is not American Club (*T. compactum*) remarkably resistant to *P. glumarum* even when bunted, yet is it not markedly susceptible to *T. caries*? Again, Rivet (*T. turgidum*) although highly resistant to *P. glumarum* is markedly susceptible to *T. caries*, and White Odessa (*T. vulgare*) that is markedly resistant to *T. caries* (under certain conditions) is 100 per cent. susceptible to *P. glumarum.*

Summary.

1. Observations on the incidence of yellow, brown and black rust on selected wheat varieties are recorded.

2. The intensity of yellow rust on these varieties is given.

3. It is shown that bunt increases the susceptibility of varieties to yellow rust.

4. It is suggested that in testing wheat varieties for resistance to *P. glumarum* the seed should be previously bunted, and that if no yellow rust then appears that the variety should be regarded as truly resistant.

5. It is suggested that a generalisation cannot be made that the 28 chromosome group is more resistant to pathogens than the 42 chromosome group, and also that there is no firm foundation for believing that the species and varieties of wheat which are resistant to rust are resistant to bunt and *vice versa.*

In concluding these observations the writer expresses his thanks to Dr E. F. Gaines, Pullman, Washington, Prof. E. C. Stakman, St Paul, Minnesota, and Dr Mains, Perdue University, Indiana, for supplying him with a number of American wheat varieties, also to Mr F. T. Brooks, Cambridge, for his valued criticism.

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TREATMENT OF SUGAR BEET "SEED" TO PREVENT SEEDLING DISEASES

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(With Plate XXV and 3 Text-figures.)

CONTENTS.

Sugar beet "seed," as will be seen, is seldom free from disease, and it is for this reason that certain continental countries have resorted to "seed" disinfection. In England, however, since the positive or negative value of "seed" treatments for this purpose had not been tested it was thought that an investigation would be desirable. Further, since sugar beet is already an important crop in the Eastern and South Midland Advisory Provinces it was manifest that in such an investigation would be offered a local problem of first magnitude and importance.

I. The organisms responsible for blackleg in sugar beet seedlings.

It is not proposed to describe the fungi responsible for infection of "seed" but rather to show the value, or otherwise, of certain methods which have been adopted for the disinfection of the "seed." The fungus which infects the "seed," and which is said to be mainly responsible for a poor "plant" and death of seedlings in the field, is *Phoma Betae*, the symptoms produced being commonly described as Blackleg. The identity of this fungus was confirmed in 1927 when cultures made from English sugar beet seedlings were compared with cultures of *Phoma Betae* from Dutch seedlings and were found to be identical. Although there are other fungi, such as *Pythium de Baryanum* and *Aphanomyces laevis*, both of which as inhabitants of the soil may cause Blackleg, the general symptoms produced on the young seedlings by these organisms are thought to be similar and are first exemplified by water-soaked spots on the young stem at, or just below, soil level; the lesions so made increase in size, become brown, then black in colour and, by finally cutting off the water channels, cause rapid wilting and death. If such plants are carefully dug up and examined, a black constriction at soil level is apparent, and generally the young infected root presents a characteristically threadlike appearance. If the plant is older and more vigorous when attacked it may recover; in this case the diseased tissue may be confined to the outer layers until it is finally thrown off, leaving a typical scar.

Attack by the Mangold Pigmy beetle (*Atomaria linearis*) must not be confused with disease caused by Blackleg organisms (Plate XXV, figs. 1 and 2). Characteristic *Atomaria* damage is distinguishable from Blackleg by the small excavations that the beetle "bites out" from the seedling. This difference is well illustrated in Plate XXV, figs. 1 and 2. It is probable, however, that seedlings attacked by the Pigmy beetle may succumb to Blackleg, since spores may gain entrance to the tissues through the wounds that have been formed.

The infection of seed-clusters on stecklings or seed-plants occurs as the former are ripening. Such infection is preceded by the presence of spots and stripes, caused by *Phoma Betae*, on the branches and main stem; and, on these affected places, and afterwards on the seed-clusters, numerous black pycnidia are developed. Moreover, because of the large area exposed by the clusters owing to their irregular shape, and because of their absorbent nature, a favourable substratum is provided, not only for the initial establishment of *Phoma Betae*, but possibly for the collection of a heterogeneous assortment of organisms.

It seems probable that to some extent *Phoma Betae* is carried on from year to year by pycnidia on the "seed," and that infection of the seed-clusters follows, in natural sequence, various forms of plant infection, such as Blackleg on seedlings and the decay of older roots associated with *Phoma Betae*. The latter sometimes results from attack by the physiological disease, Dry Rot.

II. Some considerations of damage.

An accurate estimation of damage caused by infection of seed-clusters is difficult, but field experience has shown that it may be very severe. In the field the loss of "plant" is seldom noticed unless some patches or large portions of the field are barren, and as this frequently occurs, resort is often made to re-seeding or sowing to swedes. It is not only this obvious loss which should be considered, but the annual loss which is usually undetected and which is due to the failure of many of the sprouted seeds to show above ground. This loss is not brought to light by ordinary germination trials, because the exact count of the germinated "seeds" does not indicate the proportion of these which will have vigour enough to throw off the disease and appear above ground. Trials, discussed later, have shown how large is the proportion of seedlings which are prevented from showing above soil level and of which no indication is given in figures representing the percentage of germination. It was thought that if by the treatment of "seed" such losses caused by the presence of disease on the clusters could be avoided or be reduced to a stable level, a great step towards assuring an optimum "plant" establishment in the field would have been made. Neither too thin nor too thick a "plant" is desirable prior to singling; too thin a "plant" means a resulting crop below maximum, and too thick a "plant" extra labour in singling and also a lighter crop due to the decreased vigour of the young plants which are allowed to remain. The optimum establishment is therefore a vital factor determining the final yield. By repressing

Blackleg the number of stunted roots of low sugar content would also be reduced, and, in addition, the saving of "seed" which would be effected would be a desirable economy.

III. Preliminary trials with disinfectant "seed" treatments.

(a) *Trials*, 1926.

In 1926 eight materials, namely, water, $\frac{1}{2}$ per cent. and 1 per cent. mercuric chloride, 1 per cent. acetic acid, 1 per cent. and 2 per cent. carbolic acid, two organic compounds containing mercury, and a proprietary dust, were selected from a large number of recommended treatments to determine whether any of these increased the percentage of germination in the field or accelerated germination. Although it was found that none of the treatments accelerated germination in the field, an ortho-chlor-phenol mercury material[1], and a 2 per cent. solution of carbolic acid[2], both used as steeping solutions, increased the "plant" over that of untreated "seed."

(b) *Greenhouse trials*, 1928.

As the results of the above trials referred only to a "seed" sample, the percentage of infection of which was not determined, trials were carried out in early 1928, using "seed" of which the percentage infection was known.

Table I.

Showing the mean percentage difference of the number of plants resulting from treatment and no treatment in greenhouse trials.

Treatment	Mean (no. of plants)	Measure of significance of means	Mean percentage difference from untreated
Untreated (control)	55±4·4	—	—
Mercury material*	76±5·2	3·09	+38
Ortho-chlor-phenol mercury	66±1·8	2·31	+20
Copper carbonate and mercuric chloride	44±5·4	2·01	−20

* A proprietary material the active constituent of which is said to be cresolsodium mercuric cyanide, containing about 17 per cent. of mercury. It is applied at the strength of $3\frac{1}{8}$ per cent., 1 quart being used to $12\frac{1}{2}$ lb. of "seed." The "seed" is then spread out on the floor or put in a mixing machine or agitator and mixed for three minutes. It is then spread out on the floor to dry partially before placing in the drill.

[1] A proprietary material the active constituent of which is said to be ortho-chlor-phenol mercury, containing about 17·4 per cent. of mercury. The strength at which it was used was 0·25 per cent., and in this diluted solution the "seed" was steeped for 2 hours.

[2] "Seed" was steeped for two hours.

For this purpose an ordinary commercial sample was obtained and this was examined by the Official Seed Testing Station, Cambridge. As the result of tests[1] it was found that 32·9 per cent. of the germinable seed-clusters produced growths showing disease lesions. Trials were then set out in a heated greenhouse in a large bed of unsterilised, fresh, garden soil. The means with their standard errors together with the measures of significance are given in Table I.

In Table I the significant increase given by the mercury material[2] should be noted.

In addition to these treatments four others were tried, namely, copper carbonate powder, a proprietary dusting powder, the latter powder mixed in equal proportions with copper carbonate, and the Dutch warm copper sulphate treatment[3]. The results obtained in these cases were not significant.

Estimations were made in all cases for Blackleg, but no significance could be attached to the figures obtained. The reason for this is manifest from the results of later experiments, in which it was found that the majority of the seedlings from untreated "seed" did not appear above ground; in these preliminary experiments this factor was not taken into consideration. It must be stated that in recording increase in "plant," only the last of five readings, taken at intervals between March 1st and July 3rd, has been taken as a basis for estimating the results. These readings, recorded for all treatments during the period mentioned, remained relatively constant. Since the minimum amount of water was given to the bed throughout the period of the experiment, the value of the mercury material for "seed" treatment under dry conditions for germination and establishment of the plants in the field seemed thus to be indicated.

Further greenhouse trials were carried out at Cambridge, and in each case the treated "seed" gave a significant increase of "plant" over the untreated.

(c) *Preliminary field trials*, 1928.

A parallel set of trials, using similar "seed" and similar treatments, was conducted at Oxford in the field, in duplicate plots. The "seed" was sown on April 3rd and counts were taken one month later. The means, σ, and odds as calculated by "Student's" method[4] together with the mean percentage of increase are given in Table II.

[1] Table VI. [2] See footnote *, p. 545.

[3] See Attack on sugar beet and mangold by *Phoma Betae*. *Verslagen en Mededeelingen van den Plantenziektenkundigen Dienst te Wageningen*, No. 47.

[4] *Biometrika*, VI, 1–25, and XI, 414–17.

Table II.

Showing actual mean increment in number of plants resulting from treatment together with σ, odds, and mean percentage increase.

Treatment	Actual mean increment in no. of plants resulting from treatment	σ	Odds	Mean percentage increase
Mercury material*... ...	317	6·0	165	193
Proprietary powder ...	177	12·7	42	108
Ortho-chlor-phenol mercury	115	1·0	344	70
Copper sulphate (warm) ...	86	34·6	78	52

* See footnote *, p. 545.

Treatment with the mercury material resulted in a significant increase of "plant" of 193 per cent., and the proprietary dusting powder significantly increased the "plant" by 108 per cent. The latter substance thus gave results of significant value in the open in contrast to those obtained in the greenhouse.

In this trial, as in the former, warm copper sulphate was less efficient than the mercury material. For this reason, together with the fact that, for steeping at the exact stipulated temperature, the former treatment would necessitate special costly machinery and would also require apparatus for drying the "seed" afterwards, no further trials were made with it. The ortho-chlor-phenol mercury material also showed to better advantage in the field than in the greenhouse, but further trials with this were discontinued because the other material containing mercury proved superior in these trials.

Of the three remaining treatments, neither copper carbonate nor a substance containing a small percentage of mercuric chloride mixed with copper carbonate, nor a mixture of equal parts by weight of copper carbonate and the proprietary dusting powder, significantly influenced the "plant."

The "seed" used in the above trials was then replaced by an ordinary commercial sample obtained from the Eynsham Sugar Beet Factory, and was sown in the open in Oxford on May 14th after being treated with mercury material by the sprinkle method. The results obtained by "Student's" method from triplicate plots giving the respective means, σ, and odds together with the mean percentage of increase are given in Table III.

It is seen that by June 6th the "plant" had been increased by this treatment by 30 per cent.; by July 13th the significant increase over the untreated sample was 39 per cent. The percentage that showed typical Blackleg symptoms on lifting was 45 per cent. in the case of the treated

Table III.

Showing actual mean increment in number of plants resulting from treatment together with σ, odds, and mean percentage increase.

Treatment	Actual mean increment in no. of plants resulting from treatment	σ	Odds	Mean percentage increase
Mercury material*				
1st count June 6th ...	93	5·5	52	30
2nd count July 13th ...	115	2·5	144	39

* See footnote *, p. 545.

and 5 per cent. in the case of the untreated sample. Although the latter results appear contradictory, it may safely be assumed, as a later trial indicates, that a large number of diseased seedlings remained below ground in the control, whereas the treatments allowed many of the attacked seedlings to appear which otherwise would have been prevented from doing so because of attack by the seed-borne fungi. It will also be seen from these trials that given favourable conditions for the development of the disease on the seed-clusters a high loss of "plant" is possible when an ordinary "seed" sample is sown.

(d) *Analysis of all seedlings above and below soil level.*

An analysis of all seedlings, above and below soil level, derived from untreated seed-clusters from the same sample of Kühn "seed" used earlier, is given in Table IV.

Table IV.

Showing the number of seedlings with Blackleg that originated from untreated "seed."

Total seed-clusters	Total not viable	Remaining below soil level		Appearing above soil level	
		Clean	Blackleg	Clean	Blackleg
723	39	397	566	62	57

The "seed" in this experiment was sown in large boxes in unsterilised soil in a greenhouse and analyses were made ten days after sowing. The greenhouse and soil conditions were ideal for fungal development in regard to moisture and heat. It can be seen that 59 per cent. of the seedlings which remained below ground showed symptoms of Blackleg.

(e) *Is the beneficial effect of wet treatment due to the influence of water or the chemical?*

As it was desired to prove whether the beneficial effects of wet treatments might be due entirely, or partly, to their wetting properties, the influence of water on the seed-clusters was investigated. Accordingly, "seed" of the variety Kühn, as used formerly, was wetted with water, at the same rate of application as that used in applying a solution of the mercury material; its influence was then compared with that produced by a solution of the mercury material applied at the normal rate. Both treatments were compared with controls which received no treatment and were sown in a dry state.

Each row in this trial contained 50 seed-clusters and each treatment comprised twenty rows, *i.e.* there were 1000 clusters altogether. They were set out in boxes of unsterilised soil in a greenhouse. It was arranged that each row of clusters receiving the water treatment was situated between one treated with the mercury material and another not receiving treatment. Twelve days after sowing, the number of plants which appeared in each row was counted. The results with there respective means, measures of significance, and standard errors together with the mean percentage increase are given in Table V.

Table V.

Showing the mean number of plants obtained from "seed" untreated, treated with water, and treated with the mercury material respectively, together with percentage of increase by treatment.

Treatment	Mean	Measure of significance	Percentage increase
Mercury material* ...	38·0±2·80	5·16	90
Water	24·0±3·01	1·09	20
No treatment ...	20·3±2·08	—	—

* See footnote *, p. 545.

The increase of "plant" over that from untreated clusters, the significance, as may be seen, being very great, was 90 per cent. as the result of treatment with the mercury material, whereas water alone did not significantly affect germination, the measure of significance being 1·09. It seems conclusively shown that water was not the active constituent of the mercury material which had increased the "plant" in these trials.

(*f*) *Estimations in the laboratory of the percentage of diseased growths which arose from treated and untreated "seed."*

Prior to the laying out of the more extensive field trials, "seed" of the variety Kühn, taken from the same sample as that formerly used, and treated respectively with (1) carbolic acid, ½ per cent., steeped for half-an-hour, (2) the mercury material[1], 3⅛ per cent., sprinkled on the "seed," (3) the proprietary dusting powder, and (4) untreated, was examined at the Official Seed Station for (*a*) the total diseased growths arising from clusters, (*b*) the number of normal growths, and (*c*) the number of clusters which did not germinate. Two hundred clusters from each treatment were pressed into moistened, sterilised sand, and a periodical examination was made during the germination trials. Ten seed-clusters were sown in each container of which there were twenty for each treatment. The results of the first, second and third tests conducted during the periods (1) Sept. 15th to Oct. 3rd, (2) Nov. 6th to Nov. 13th and (3) Nov. 15th to Nov. 23rd respectively, are given in Table VI.

Table VI.

Showing the percentage of diseased growths which resulted from treated and untreated "seed" of the variety Kühn.

Treatment	Test no.	Average percentage germination	Percent. of germinated clusters having diseased growths	Average percent. of clusters with diseased growths in tests nos. 2 and 3
Carbolic acid, ½ per cent.	1*	85·5	94	32·1
	2†		16·5	
	3†		47·7	
Proprietary dusting powder	1*	91·5	73	8·5
	2†		7·5	
	3†		9·6	
Mercury material ...	1*	87·5	35	6·2
	2†		8·4	
	3†		4·1	
Untreated	1*	87·5	96	32·9
	2†		15·1	
	3†		50·8	

* In test No. 1 conducted during the period Sept. 15th to Oct. 3rd only diseased growths were removed from the dishes, the remaining growths were left until the experiment terminated, so that a large proportion of the latter undoubtedly contracted infection through the diseased growths contaminating the sand.

† All germinated clusters were removed from the dishes on the 8th and 14th days.

It may be seen that the mercury material in tests No. 2 and No. 3 was the most effective in reducing the average percentage of germinated

[1] See footnote *, p. 545.

clusters having diseased growths. It is also of interest to note the wide fluctuations in these percentages in the carbolic acid and untreated samples. The high percentage in test No. 1 is explicable since all growths were not removed at regular intervals as in tests Nos. 2 and 3. It is probable that in the second and third tests the temperatures were different, favouring the parasite or parasites in one case, and in the other case retarding them. It is seen that ½ per cent. carbolic acid was not effective in reducing the percentage of diseased growths. The proprietary dust on the other hand was quite effective in this regard.

(g) *Preliminary trials on the treatment of "seed" with sulphuric acid and comparisons with treatment with the mercury material.*

Sulphuric acid at strong concentrations has been used for the purpose of "decorticating" seed-clusters of sugar beet. This treatment effects a corrosive action on the exterior of the clusters, and as the result of carbonisation they become darker in colour and are considerably reduced in size. The treatment also serves to break up the clusters and thus allows the drill to distribute the "seed" more evenly and efficiently. The effect of the treatment as a disinfectant against *Phoma Betae*, and indirectly for the purpose of increasing "plant" establishment brought about entirely through its disinfecting action or partly through its action in stimulating the "seed," has received some attention during these in-investigations[1].

In 1929, field trials were conducted at Oxford and Cambridge which were preceded by a preliminary test conducted in a greenhouse. The following treatments were employed: water, an organic compound containing mercury, 60 per cent. sulphuric acid, and the latter treatment followed by treatment of the dried "seed" with the organic compound just mentioned. The acid treatment consisted in steeping the "seed" in a 60 per cent. solution of sulphuric acid for a period of 45 minutes. This procedure was followed by submerging the "seed" in a large volume of water, so that thereby further action of the acid was prevented and, at the same time, disastrous results to the "seed" avoided which, in the event of a small volume of water being used, would occur owing to the evolution of heat. After washing in running water for several hours the "seed" was neutralised with a 1 per cent. solution of soda until litmus paper showed no change of colour. After drying the "seed" was ready for sowing. As the result of trials in the greenhouse it was

[1] Acknowledgment is made to Dr R. M. Woodman, Cambridge, for information concerning this treatment.

found that the mercury material mentioned allowed the greatest number of plants to be produced. The next treatment, in order of number of plants produced, was the combined acid and mercury treatment, and lastly came untreated "seed" and "seed" treated with water. Trials were then conducted outside, and an examination of the plots was made for the number of plants arising as the result of each of the treatments. The "seed" was sown on May 9th at Cambridge and on May 11th at Oxford in rows eight feet in length and six inches apart. Each treatment occupied one row and each contained 100 clusters sown by hand. Each treatment and the controls were replicated twelve times and were arranged in a randomized order in each series. The following table shows the mean number of plants resulting from each treatment.

Table VII.

Showing the mean number of plants resulting from each treatment.

Treatment	Mean no. of plants	
	A series	*B* series
Sulphuric acid	15	38
Mercury material*	37	59
Sulphuric acid and mercury material	31	50
Water	17	34
Untreated	21	31

S.E. of mean difference in Series *A* is 4·17 and in Series *B* 5·63.
S.E. ×2·57 in Series *A* is 11 and in Series *B* 14.

* See footnote *, p. 545.

Series *A* and *B* represent plots at Oxford and Cambridge respectively. The data have been analysed by the method utilising the average variance for any two plots[1]. The standard error of mean difference based on twelve pairs of plots is 4·17 in Series *A* and 5·63 in Series *B*. Any difference equal to or exceeding the figure obtained by multiplying the standard error by 2·57 will have a significance of 100 to 1. These figures are for Series *A* 11 and for Series *B* 14.

The difference between the mean number of plants in the water and untreated plots is less than the standard error in both Series *A* and in Series *B*, so that these treatments are not significantly different. It should also be noted that the mean differences in the case of plots treated with the mercury material, and those treated with sulphuric acid fol-

[1] *The Principles and Practice of Yield Trials*, F. L. Engledow and G. Udny Yule. Published by the Empire Cotton Growing Corporation, Millbank, London, S.W. 1, from vol. III, Nos. 2 and 3, of *The Empire Cotton Growing Review*.

lowed by mercury material, are not significant in both series. The sulphuric acid and untreated plots do not significantly differ.

The mercury material used either as an additional treatment after sulphuric acid, or alone, was responsible for a greater number of plants than in the case of any of the other treatments, and the mean difference obtained was the only significant result. The increases resulting from the mercury material were 70 per cent. and 97 per cent. respectively in Series *A* and *B*.

IV. Comparative field trials with three "seed" treatments.

Treated "seed" with untreated as controls was sown in field plots at the Cambridge University Farm and at the Farm Institute, Moulton, Northants. At Moulton the soil is light and sandy on ironstone formation, and the area which the plots covered had received similar treatment in cropping and manuring for many years. The "seed" was sown at the rate of 15 lb. per acre on May 3rd and 4th, 1928, using a five coulter drill; a strip of seven chains and composed of five drill-rows comprised each plot treatment. It was desired to find the effect of the dressings on:

(*a*) "Plant" establishment.

(*b*) Vigour of the seedlings as judged by dry and green weights, and the percentage of the plants attacked by Blackleg.

(*c*) Yield, and the percentage of sugar.

(a) *Field trials to estimate the "plant" establishment and percentage of Blackleg as the result of three "seed" treatments.*

The "seed" in the different plots showed little or no difference in their times of germination. The counts of the number of plants from ten one-yard strips were taken on June 8th immediately previous to singling; the strips were removed from the five drill-rows geometrically, in such a way that statistical analysis for the standard error was applicable to the results of the readings for each of the plots.

Table VIII.

Showing for each treatment the mean number of plants with its standard error. The mean number of plants showing Blackleg is also included.

	Mercury material	Proprietary dust	Carbolic acid ½ per cent.	Untreated
Mean no. of plants	45±5·46	19±2·86	25±2·12	20±2·28
Mean no. of plants with Blackleg...	10	7	4	6

Table VIII gives the results from this method of examination, including in each case the mean number of plants with its respective standard error and the significance. The mean number of plants showing Blackleg symptoms is also included.

The measures of significance of the mean differences are: 4·14, 0·27, 1·16, and 2·28 respectively. The only significant increase of "plant" over the untreated was that due to the mercury material[1], and this was responsible for the large increase of 125 per cent. As before, the differences between the means in the case of plants attacked by Blackleg were not significant.

At Cambridge, estimations showed that the mercury material increased the "plant" over untreated by 83 per cent. and similarly the proprietary dust by 70 per cent. and carbolic acid by 15 per cent. in one series of plots. The differences between the means of plants attacked by Blackleg were not significant as the plants above ground only were examined.

"Seed" from the same source was used in field plots at Moulton and Cambridge, namely, a commercial sample of which the proportion of germinable "seed" producing diseased seedlings was found to be 32·9 per cent.[2] All treatments, in these two field plots, were conducted by the same person, and each treatment was carried out in bulk so as to avoid differences due to the personal factor or those due to slight variations in method.

(b) *Vigour of the plants as judged by green and dry weights of seedlings derived from the treated and untreated samples.*

The average weight, both in the green condition and after air drying to constant weight, of seedlings from seed-clusters receiving each of the three treatments respectively, were compared with the corresponding weights of seedlings from untreated "seed." It was hoped that such comparisons would give some indication of the increased vigour of the plants due to "seed" treatment. The following table gives the average weights of seedlings in the trial plots at Cambridge. All the seedlings examined for "plant" establishment were included in obtaining the results noted in Table IX.

It may be seen that there is an average increase per seedling in both dry and green weight as the result of using each of the three treatments. The mean green weight and dry weight was increased by the treatments by 12 per cent. and 7·4 per cent. respectively, and thus field observations, which are indicative of increased vigour induced by treatment, were confirmed.

[1] See footnote *, p. 545. [2] See Table VI.

Table IX.

Showing the average green weight and the average dry weight of seedlings from treated and untreated "seed" sown in the field.

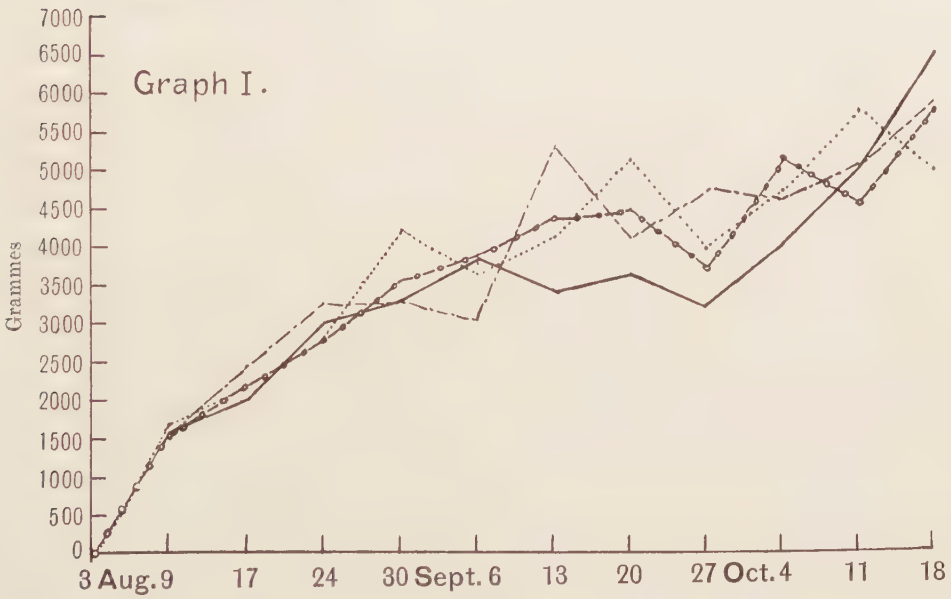
Treatment	Total seedlings	Average green weight in gm.	Average dry weight in gm.
Mercury material ...	486	0·58	0·0444
Proprietary dust... ...	610	0·58	0·0460
Carbolic acid, ½ per cent.	648	0·51	0·0445
Untreated	370	0·50	0·0418

(c) *Effect of the treatments on yield and the percentage of sugar.*

The weights of the washed and "topped" roots, and the weights of the "tops," were taken at eleven weekly intervals in the four field plots at Moulton, the first weighing being made on August 9th. Thirteen beets were taken from each plot to obtain the average weight and, as

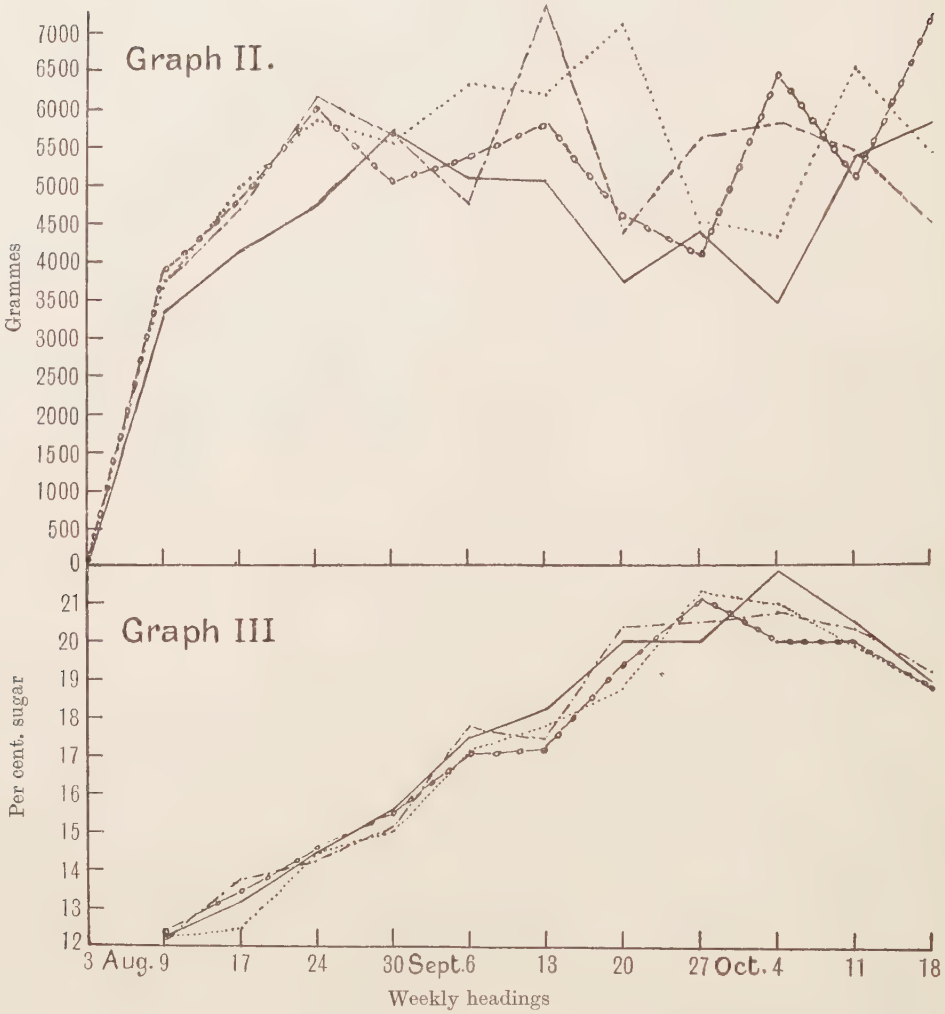
Table X.

Showing in three graphs, the average weights of the roots after washing and "topping" and of "tops," and of the mean percentage of sugar from the four plots for eleven weekly readings taken between August 3rd and October 18th.



Graph I. Showing average weight of washed and "topped" roots in grammes.

Table X (*continued*).



Graph II. Showing average weight of "tops" in grammes.
Graph III. Showing mean per cent. of sugar.

each beet was taken in such a way that the area representative of each plot was covered in obtaining the sample, an effort was made to obtain a representative average weight per beet for each of the treatments. The percentage of sugar was obtained at the same time and the results, presented in the form of graphs, are given in Table X.

In Graph I it may be seen that the weights of the washed and "topped" roots were about equal in the carbolic, proprietary dust and the untreated plots, and that these weights maintained a fairly steady upward trend. In the mercury plot the weight of the washed and "topped" roots did not significantly increase during September, but a steady upward trend is noted in October which finally slightly supersedes the weights obtained for the other three plots. As shown in Graph II, the fluctuations in the weights of the "tops" are less constant. In September a decisive drop in the weights of the "tops" in all the plots is noted and this is particularly reflected in the mercury plot in Graph I in the cessation of increase in weight during September of washed and "topped" roots. The weights of the "tops" in the final reading may be considered as about equal for all dressings if due consideration is given to the fluctuations which occurred earlier.

The percentage of sugar of the roots during this period is given for the four plots in Graph III. It may be noted that the percentage of sugar in all four plots was practically equal and maintained a steady upward trend until October. During the first week of October the percentage of sugar in each plot started at the same time on a somewhat downward trend but the end-point reached was practically identical. The decrease in the percentage of sugar was due to the rapid growth of new heart leaves during the first half of October. The highest sugar concentration occurred in the mercury material plot, namely 21·9 per cent. of sugar, in an analysis made on October 4. In summarising it may be said that there were only slight differences between roots in regard to weight of root, weight of "tops" and percentage of sugar as the result of the three treatments. The general reduction of the percentage of sugar in all treatments as the result of the growth of new heart leaves is a point of interest.

Table XI.

Showing the final yield of washed and "topped" roots, "tops" and weight of sugar together with the estimated number of roots per acre for each plot.

	Untreated	Mercury material	Proprietary dust	Carbolic acid
Weight of washed beet, in tons per acre	10·10	10·24	10·03	9·20
Weight of tops, in tons per acre ...	12·7	12·7	13·0	11·6
Weight of sugar, in tons per acre	1·93	1·88	1·93	1·79
Number of roots per acre	23,760	26,840	25,520	26,400

It may be seen that both the weight of the "tops" and the weight of washed and "topped" roots were decreased by approximately one ton

per acre through treatment with ½ per cent. carbolic acid, whereas neither the weights of the "tops" nor the weights of washed and "topped" roots were significantly affected by the mercury material or the proprietary dust. The number of beets per acre from "seed" treated with carbolic acid and from "seed" treated with the mercury material was slightly greater than the number produced in the case of the proprietary dust, and all three treatments produced a significant increase of mature roots over the number produced by untreated "seed." In the case of "seed" receiving the carbolic acid treatment a slight decrease in the weight of sugar per acre is noted. As may be seen there was no significant increase in weight of sugar per acre as the result of using either the mercury material or the proprietary dust. In summarising it may be said that ½ per cent. carbolic acid exerted an undesirable effect in reducing the weight per acre of "tops," "topped" roots and sugar. On the other hand the other two treatments did not exert undesirable effects.

V. Effect of the treatments on commercial "seed" as shown by the results of trials in which ten different varieties of "seed" were tested.

The trials so far had been limited to three commercial samples of "seed" and the beneficial results from the use of the treatments had been observed only on the particular samples chosen. It was desired to find the effect of similar treatments on commercial "seed." Accordingly 26 samples of commercial "seed" were selected from different districts in different countries, and of these ten were taken at random to find the relative effects of the treatments. The dressings used were: (1) the mercury material, (2) a ½ per cent. carbolic acid, steeping process, and (3) 3½ per cent. carbolic acid, sprinkle method. Germination tests of the ten varieties were first carried out at the Official Seed Testing Station, Cambridge, with the results given in Table XI.

(a) *Effect on germination.*

It may be seen from these figures that the effect of the treatments on the final germination was very slight, but that "seed" treated with mercury material was a little longer in germinating than other samples under the conditions of this experiment. This delayed germination has not been noted in any of the field trials.

Table XII.

Showing the percentage of germination of ten different sugar beet varieties treated and untreated.

Variety	Test no.	Mercury material % germ*		Carbolic† ½ % % germ*		Carbolic 3½ % % germ*		Control % germ	
		6 days	Final	6 days	Final	6 days	Final	6 days	Final
Sharpe's WZ ...	1	57	72	68	72	70	75	69	71
	2	72	75	—	—	—	—	—	—
Debrovice ...	1	60	73	64	69	58	72	73	76
	2	26	55	—	—	—	—	—	—
Hilleshog	1	65	82	70	77	83	88	72	80
Braune	1	75	85	90	93	91	94	90	93
Dippe	1	81	89	82	84	83	86	76	78
Kleinwanzleben ...	1	70	75	74	77	69	75	70	73
	2	44	69	—	—	—	—	—	—
Kühn	1	74	88	81	86	84	88	84	88
Schreiber	1	55	81	80	81	77	82	72	75
Rakovsky	1	71	83	84	86	87	89	86	88
Zapotil	1	49	69	72	79	69	78	71	78

* *I.e.* percentage of clusters each giving at least one seedling.

† One quart to 12½ lb. of "seed."

(b) *Effect on "plant" establishment.*

"Seed" from the same treated and untreated sample lots was sown in Oxford in a well prepared, even, friable seed-bed on July 6th and 7th, 1928. Of each of the three differently treated samples of "seed" of the ten chosen varieties two hundred clusters were sown by hand in rows

Table XIII.

Showing the mean difference from untreated, σ, the odds and the mean percentage difference.

Variety	Treatment	Mean difference	σ	Odds	Mean per cent. difference from untreated
Dippe	Mercury material*	106	54·8	103	+48
Debrovice ...	Mercury material*	61	21·2	434	+30
	Carbolic 3½	50	12·7	1427	+27
Hilleshog ...	Mercury material*	88	49·1	85	+46
Zapotil	Carbolic ½	72	19·2	1101	+36
	Mercury material*	23	20·6	20	+12
Kleinwanzleben	Carbolic 3½	44	27·5	60	−24
Braune	Carbolic 3½	63	54·1	23	−19
	Carbolic ½	69	37·7	824	−21
Sharpe's WZ ...	Carbolic 3½	19	4·6	1665	−12

* See footnote *, p. 545.

eight feet in length replicated five times. All the rows comprising the ten varieties were laid out in randomised fashion, but each row had an adjoining row sown with untreated "seed" from the same variety sample. "Student's" method was used to calculate the significance of the difference of means. For the sake of brevity those results not significant are not included in Table XIII.

These trials showed that four of the varieties, namely Dippe, Debrovice, Hilleshog and Zapotil, gave a positive response of significant value to treatment with the mercury material as shown by an increase of "plant" ranging from 12 per cent. to 48 per cent. The remaining six varieties, namely Kühn, Schreiber, Rakovsky, Sharpe's WZ, Braune and Kleinwanzleben were not significantly affected adversely or advantageously by this treatment.

Carbolic acid, $3\frac{1}{2}$ per cent., significantly increased the "plant" of Debrovice by 27 per cent. but significantly decreased the number of seedlings in the three varieties Kleinwanzleben, Braune and Sharpe's WZ by 24, 19 and 12 per cent. respectively. The remaining varieties were not significantly influenced by the $3\frac{1}{2}$ per cent. carbolic acid treatment.

One variety, Zapotil, gave a significant increase of "plant" of 36 per cent. after being treated with $\frac{1}{2}$ per cent. carbolic acid, but the same treatment significantly decreased the "plant" of Braune by 21 per cent. The remaining eight varieties treated with $\frac{1}{2}$ per cent. carbolic acid were not significantly affected.

A long period of drought followed sowing, and this was undoubtedly responsible for reducing the obvious effects of treatment on establishment of "plant," but, even so, one of the treatments, namely that using the mercury material, was distinctly beneficial to four varieties and did not disadvantageously affect the remainder. Steeping "seed" in $\frac{1}{2}$ per cent. carbolic acid, which is a very old specific treatment on the Continent for sugar beet, and also a modified form of this using the acid at a strength of $3\frac{1}{2}$ per cent. for sprinkling on the "seed," proved of little value under these conditions; the latter significantly decreased the "plant" of three varieties, and neither of the treatments was responsible for a general improvement of "plant."

At Cambridge similar plots were laid out and field observations indicated that no obviously adverse effect had followed the treatment of "seed."

(c) *The relationship of the percentage of germination of the "seed" and percentage of germinated seed-clusters producing diseased growths to the mean percentage of increase in the number of plants in response to treatment.*

The ten varieties used in these trials were then submitted to the Official Seed Testing Station to be examined for diseased growths. The results are given in Table XIV.

Table XIV.

Showing the percentages of germination of diseased growths, and of increase in the number of plants as the result of treatment with the mercury material.

Variety	Per cent. of germination	Per cent. of germinated clusters with diseased growths	Percentage increase of "plant" by treatment*
Braune	94·5	95·0	—
Rakovsky ...	91·0	95·0	—
Dippe	88·5	90·5	48
Schreiber	83·0	94·6	—
Hilleshog	80·5	91·3	46
Debrovice ...	83·5	84·5	30
Kleinwanzleben ...	95·5	90·7	—
Kühn	88·0	77·4	—
Zapotil	84·5	75·0	12
Sharpe's WZ ...	78·0	57·0	—

* Table XIII.

Each of the results in the first two columns was obtained after the examination of 200 clusters which had been germinated in sterilised sand. As in previous trials[1] all growths were removed as they appeared. Firstly, it may be noted that there is no correlation existing between the percentage of germinated clusters which produced infected growths and the percentage of germination. Secondly, this examination had shown how high is the percentage of infected growths which develop from "seed" of the majority of commercial varieties. Concerning the four varieties influenced by the mercury treatment in a former trial[2] there is a direct correlation between the percentages of clusters producing diseased growths and the percentages of increase of "plant" by this treatment.

[1] See Table VI and following paragraph. [2] Table XIII.

It appears, then, that the greater the intensity of the disease in a sample the greater is the beneficial action of the treatment. An inference which may be drawn from this fact is that, although the percentage of infected growths as obtained from these trials does not necessarily correspond with that proportion of seedlings seriously handicapped or killed by disease, this percentage does serve to indicate the relative intensity of attack controllable by treatment. It is obvious, then, taking two extremes encountered in these trials, that, if so large a proportion as 95 per cent. of the germinable clusters of a certain variety produced diseased growths, the intensity or pathogenicity of controllable disease is greater than it is in the case of a variety in which 57 per cent. of the "seed" produced diseased growths. This aspect of attack should be considered in interpreting these results. For example, the relatively small increase of "plant" of 12 per cent. in the case of Zapotil "seed," infected to the extent of 75 per cent., may be explained by the hypothesis that the lethal action of the disease in this case would be much less prominent than it would be in the case of Dippe "seed" infected to the extent of 90·5 per cent. This was actually shown to be the case as it can be seen that the response of the latter to treatment was much more marked as shown by a significant increase of "plant" which was 48 per cent. greater than the "plant" derived from untreated "seed."

(d) *Preliminary observations relating to commercially supplied "seed" treated with the mercury material.*

In co-operation with a number of sugar beet factories endeavour was made to make as many observations as possible in the field where treated[1] "seed" supplied commercially was being sown. Such endeavours in nearly every case were unfruitful. It was found that no observations were of value unless the lay-out of plots and supervision of all details concerning the soil, sowing of the "seed," etc., were in the hands of an experienced officer. It was found that the number of factors, any of which could bias the results of observations, was very great.

On the Cambridge University Farm an effort was made to compare the respective number of plants resulting from untreated "seed" and treated "seed" purchased as such. The "seed" was sown at the same rate per acre by means of a drill. Sampling was accomplished by selecting ten representative strips of the drill-row 50 feet in length, in such a way as to cover representatively the area occupied by each treatment. The

[1] Treated with the mercury material, cf. footnote *, p. 545: method of treatment not known.

mean number of plants in each case, together with the measure of significance and standard errors, are shown in Table XV.

Table XV.

Showing the mean number of plants resulting from commercial "seed" untreated and treated. The percentage of Blackleg is also given.

Treatment	Mean number of plants	Per cent. of Blackleg	Average per cent. increase by treatment
Untreated	70 ± 6·9	8·7	—
Mercury material ...	99 ± 14·4	4·4	41·5

The measure of significance of the difference of the means is 1·85, thus being barely significant. The average increase of 41·5 per cent. should be noted. The average dry weight per seedling was found to be 0·0488 in the case of untreated "seed" and 0·0530 in the case of treated "seed," *i.e.* an increase of 8·6 per cent. In a former test it will be noted[1] that the average increase in dry weight per seedling as the result of the three treatments was 7·4 per cent. In the table to which reference is made it will also be noted that the increase by the mercury treatment was 6·2 per cent. per seedling.

It will be necessary to make more extended tests of treated "seed" offered to growers. It is suggested that "seed" is not always as efficiently disinfected in bulk as in the carefully supervised treatment of relatively small quantities. For this reason "seed" treated in the course of these investigations does not compare with treated "seed" from suppliers. It is also suggested that the results obtained from such "seed" would be incomparable for another reason, namely, moisture content. "Seed" treated under personal supervision and used in these trials was sown when relatively wet. The standard to be aimed at was unhindered delivery by the drill and the avoidance of inconvenient clogging. Data[2] are to hand which show that in many cases only partial disinfection was attained as shown by the examination of treated "seed" offered to growers. The results of the examination for the presence of *Phoma Betae* together with relevant data are shown in Table XVI.

In addition to the samples, to which reference is made in the following table, four others representing three well-known varieties were examined and found to be infected by *Phoma Betae* to a negligible extent, so that re-treatment was not undertaken. To obtain the percentages in the last

[1] Table IX and preceding paragraph.

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Table XVI.

Showing the percentage germination and infection of five samples of commercial treated "seed"; also the percentage increase in the number of growths and decrease in the number of diseased growths after re-treatment.*

Variety	Per cent. germination	Per cent. infection	Per cent. increase in the number of growths after re-treatment	Per cent. decrease in the number of diseased growths after re-treatment
A†	86	50	21	95
B	87	25	8	82
C	84	36	46	75
D	—	34	24	92
E	90	51	24	67

* A $\frac{1}{2}$ per cent. solution of the mercury material was used in which the "seed" was steeped for two hours.

† *A–E* represent samples of four common varieties.

two columns 100 clusters were allowed to germinate and the total number of growths which appeared was compared with that obtained from "seed" as supplied in its original treated state. It can be seen that there is a great variation in the percentage of infection of treated "seed" as received from suppliers. The efficacy of re-treatment is shown.

VI. Discussion.

It is highly desirable that the "plant" should not fail in the field when circumstances favour seedling diseases, as they do in certain years, and it cannot be denied that it is a wise precaution to insure against such losses which, although they may be of little consequence one season, might be of vital importance in another. Furthermore, the small cost and little trouble which is required in the application of one of the "seed" treatments are of small account when compared to the advantages which may be derived. It cannot be said that such treatment of "seed" would result in an increased yield or an increased percentage of sugar in any one season, but there is always the possibility that under conditions favourable to seedling diseases the yield would be increased.

It seems probable that the optimum "plant" would be established in the field if it were possible to judge more accurately the amount of "seed" to be sown per acre; this, assuredly, would be made more simple by using either disease-free "seed" or treated "seed," as then much would be accomplished towards assuring an even "plant" by lessening the effects of that variable factor the infection of "seed." The optimum

"plant" as already mentioned, is of considerable importance, for it must be pointed out that too thick a "plant" as well as too thin a "plant" must both exert an adverse effect on yield. The real although less obvious effect of too thick a "plant" is to reduce the vigour of the plants left after singling by previously crowding together the seedlings in the row.

In conclusion, a reference to the views expressed in a recent paper by Engledow and Maher is appropriate in pointing out the importance of obtaining a full and even plant population. Their conclusions are as follows: "It is in sugar beet, of all our field crops, that a full plant is to be expected. In common with other root crops it is singled, only the most suitable fields are selected for it, preparatory cultivations and making of the seed bed are done with great care, and it is a spring crop. Further, it will be shown in later passages that the Continental insistence on full even plant as a guarantee of maximum yield is strictly applicable to our own conditions[1]." And again, "But the new crop is still on trial. It has to bear the grievous burden of a disappearing subsidy. Greater yield per acre is its only hope, and this, it is believed, is to be realised mainly through a full and even plant[2]."

VII. Summary.

1. The organisms responsible for Blackleg in sugar beet seedlings have been referred to and the damage that they do considered.

2. It has been shown that treatments used to disinfect the "seed" may be harmful, they may exert no influence, or they may be of considerable value.

3. The present trials have shown the beneficent effects of an organic compound containing mercury. This material increased the "plant" in the majority of cases, and this increase varied between 12 per cent. under some conditions and in increasing amounts up to 193 per cent. under more favourable conditions.

4. An increase in the vigour of young plants was noted occasionally. Quantitative tests showed an increase in both the green and dry weight per seedling as the result of treatment. The beneficial action of treatment is not due to the influence of water.

5. The organic compound containing mercury was the most effective in reducing diseased growths during laboratory tests; the proprietary

[1] Engledow, F. L. and Maher, C. A. "Yield and plant population in sugar beet." *Journ. Agri. Sci.* xviii, pt 4 (Oct. 1928), 578.

[2] *Ibid.* p. 590.

dust was nearly as efficient. Steeping in a $\frac{1}{2}$ per cent. solution of carbolic for half-an-hour was ineffective.

6. Treatment of the clusters with 60 per cent. sulphuric acid did not appreciably improve "plant" establishment, but this treatment followed by the mercury treatment increased the number of plants considerably.

7. Field trials conducted for one season on a field scale have not shown an increase in the percentage of sugar as a result of the treatment of "seed," but a slight increase in yield and in the total number of roots per acre was noted as the result of treatment with the organic compound containing mercury. The proprietary dust was not noted as being responsible for reducing the "plant" in the field. Its beneficent action was not as uniformly apparent as that of the mercury material.

8. Carbolic acid at a strength of $\frac{1}{2}$ per cent. was definitely injurious when used for steeping purposes. Furthermore, trials show no benefit from a modified form of this treatment in which a $3\frac{1}{2}$ per cent. solution is sprinkled on the "seed." Other materials tested were found to be of doubtful value.

9. Certain samples of commercial "seed" as supplied treated with a mercury material were found to be infected to a considerable extent by *Phoma Betae*. Such "seed" it was found could be efficiently re-disinfected by careful re-treatment with marked beneficial results.

In conclusion it is desired to thank those who gave assistance. Among these are Mr Alfred Eastham, Director of the Official Seed Testing Station, Cambridge, Dr Van Poeteren, Wageningen, Mr G. R. Clarke, Advisory Chemist, and Dr N. Cunliffe, Advisory Entomologist both of Oxford, Mr W. A. Stewart and Mr W. R. Seward, Dr Pethybridge and the officials of many beet sugar factories who lent a helping hand.

EXPLANATION OF PLATE XXV

Fig. 1. Blackleg damage.
Fig. 2. Pigmy beetle damage.

(*Received April 7th*, 1929.)

Fig. 2.

Fig. 1.

WOODWARD & DILLON WESTON.—Treatment of Sugar Beet "Seed" to prevent Seedling Diseases (pp. 542–566).

STUDIES IN BACTERIOSIS. XVI

THE AGGLUTINATING AND PLASMOLYTIC ACTION OF THE SAP OF THE POTATO ON VARIOUS PARASITIC AND SAPROPHYTIC SPECIES OF BACTERIA

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(With 2 Text-figures.)

In recent work on plant immunity the tendency has been to emphasise the mechanical defence against invading organisms which a plant possesses, due to its cell walls and cuticle, and also to its power to cut off the diseased portion of its tissues by rapid cork or gum formation without serious detriment to the plant as a whole. But it is evident that the susceptibility of a plant to attack, or its power of resistance, must often depend on delicate reactions between the cells of the host and the attacking organism which are difficult to detect. In the animal body the flocculation of bacteria by agglutinins in the blood is a delicate reaction of this nature, indicating some conditions unfavourable to the invader. The evidence for the presence of agglutinins in plant juices is on the whole unsatisfactory. In several cases specific agglutinins, which appear after invasion by bacteria, have been detected, but the evidence for permanent natural agglutinins is extremely scanty. The specific agglutinins are usually localised in the tissues near the infected area. Schiff-Giorgini (14) showed that a water extract of the cortical tissues of *Olea europaea* adjacent to a tumour caused by *B. oleae* (*B. savastanoi*, E.F.S.) possessed agglutinating and bactericidal properties, which were considerably greater in amount than those possessed by a water extract of healthy tissues. Kritchewsky (7) demonstrated the presence of precipitins and agglutinins in the juice of *Cotyledon Scheideckeri* after inoculation with *B. typhi* and *B. cholerae*, and Korinek (6) obtained marked agglutination with the juice of *Beta vulgaris* infected by *B. tumefaciens*, but regarded it as due to absorption on crystals and coagulated colloids of the sap, and not true agglutination. Wagner (15), too, observed the agglutination of *B. vulgatus*, *B. putidum* and *B. astero-*

sporus by the juice of the potato into which they had been inoculated. Vigliano is quoted by Korinek (6) as having found natural agglutinins in the sap of many plants, but says that Carbone denies the formation of any agglutinins in the sap of the potato, even after infection with either parasitic or non-parasitic species of bacteria.

It will be evident from the experimental work recorded in the following pages that these conflicting results are probably in part due to the fact that more delicate methods than those usually employed are needed to detect agglutination in plant juice, and also to the fact that the agglutinating and bactericidal reactions between the organism and plant vary with each species investigated.

In cases where bacteria inoculated into a certain host plant fail to produce parasitism of that host, and are agglutinated, this agglutination cannot be due to the H-ion concentration of the sap alone, since it has been shown by previous work (Berridge (2)) that H-ion by itself acts injuriously on various parasitic and saprophytic bacteria of common occurrence only at concentrations above *p*H 5·0–4·4; and Rea and Small (13), Clevenger (4) and others have shown that concentrations less acid than this prevail as a rule in adult plants. It seemed possible, however, that the agglutinating action might be exerted by acid combined with buffer substances in the sap, or by traces of trivalent elements such as iron or manganese present in it, since the salts of such metals have been shown by Eisenberg (5), Bechhold (1) and others to have an agglutinating action on bacteria even in extremely dilute solutions.

Phosphates, which are important buffer constituents of cell sap, are precipitated from plant juices when strong alcohol is added in excess, together with traces of salts of calcium, magnesium, iron and manganese, if present. The agglutinating effect on various types of bacteria of such a precipitate was therefore investigated as it might reasonably be inferred that any action possessed by such a solution would also be exercised by the cell sap itself.

Preparation of the precipitate and methods of experiment.

Peeled potatoes, usually of the variety "King Edward," were grated, and the mush packed as quickly as possible into a Büchner funnel over a thin layer of asbestos; the juice was drawn through by means of a filter pump, and immediately poured into five times its volume of 95 per cent. alcohol. A bulky precipitate appeared, this was allowed to settle overnight, the alcohol was then decanted off and the sediment dried at about 52° C. The precipitate was prepared in small quantities and

kept in a desiccator, and the solutions made up as wanted, since the latter tended to change on keeping. When required for tests the precipitate from about 4 c.c. of juice was dissolved in distilled water, being kept at about 52° C. for ten minutes; the reaction of this solution was 5·8–6·0, *i.e.* roughly that of the potato juice itself. The water had previously been autoclaved in order to drive off the CO_2 and reduce the *p*H to 7·0, and to eliminate contaminating organisms as far as possible from the tests. The volume of water used was half that of the juice from which the precipitate was obtained, so that on adding an equal volume of a bacterial emulsion, the total dilution of the active substance was, as far as could be secured, the same as the dilution in the juice itself. A large proportion of the sediment did not dissolve, and was filtered off before the solution was used. The bacterial emulsion was prepared from cultures on bouillon agar grown for 24–48 hours at 25° C. The growth was removed and emulsified with a platinum loop and not washed off, in order to avoid the introduction of soluble salts from the medium.

This solution of the alcoholic precipitate from potato was found to possess an agglutinating action on certain types of bacteria, but not on others. This agglutinating power was not destroyed by boiling the solution for 30 minutes, nor wholly lost after standing exposed to the air for 3 or 4 days.

The agglutinated clumps of bacteria did not as a rule sink to the bottom of the tube, but formed a pellicle at the surface of the liquid, or remained suspended in it. In many cases they were microscopic in size, and where agglutination was followed by plasmolysis, the bacteria were reduced to granules. Therefore films, usually stained with carbol fuchsin, were always made, in order to determine whether agglutination or plasmolysis had taken place; these were examined with a $\frac{1}{15}$th inch oil immersion lens, to make sure that chemical precipitates in the liquid were not mistaken for bacterial clumps. The course of the reaction was followed by making films $\frac{1}{4}$, 2 and 4 hours after the beginning of the test. The films made after $\frac{1}{4}$ hour, or earlier in cases where rapid plasmolysis rendered this necessary, served as controls, indicating the density of the emulsion and freedom from clumps. The 4-hour films furnished the results summarised in Table I, while the 2-hour films were useful in checking these results, especially in cases where plasmolysis was occurring. The comparative number and size of the clumps could be observed in the films, but many free bacteria were always present, since in the process of filming the clumps were partly broken up.

Fourteen different species of bacteria were tested, representing a variety of types:

B. carotovorus	Soft rot of carrot, turnip, etc.
B. solanisaprus	Rot of potato.
B. phytophthorus	Blackleg of potato.
B. delphinii	Leaf-spot of delphinium.
B. malvacearum	Leaf-spot of cotton.
B. marginale	Blight of lettuce.
B. pyocyaneus	Occasionally parasitic on plants and animals.
B. fluorescens liquefaciens...	Non-parasitic fluorescent type.
B. fluorescens non-liquefaciens	,, ,,
B. tumefaciens	Causing galls on plants.
B. coli	Non-parasitic on plants.
B. vulgare	,, ,,
B. mycoides	Sporing saprophytic type.
B. dendroides	,, ,,

Agglutination tests.

The 14 species above enumerated were subjected to three series of agglutination tests, using (1) the solution of the potato precipitate at *p*H 6·0, the natural reaction of the expressed juice; (2) the fresh juice immediately after filtration; and (3) the solution of the potato precipitate with its H-ion concentration varied by addition of acid or alkali.

(1) The first series of tests with the precipitate solution at *p*H 6·0 showed that it was precisely those types which can parasitise potato, *i.e. B. solanisaprus* and *B. phytophthorus*, which were least affected by the substances thrown down by alcohol from the sap. *B. carotovorus*, *B. delphinii* and *B. vulgare* were also little affected, but *B. coli*, *B. tumefaciens*, *B. malvacearum*, and *B. marginale* were distinctly agglutinated in small clumps, while the two species of *B. fluorescens*, *B. pyocyaneus*, and the sporing organisms were strongly flocculated. Broadly speaking, the parasitic forms were not influenced by the substances in the solution, or only to a comparatively slight degree, while the non-parasitic forms were strongly agglutinated.

(2) The second series of tests, in which the fresh juice was used immediately after filtration, proved that the agglutinating substances of the precipitate were active in the untreated juice, for the degree of agglutination of the various species was about the same as in the previous tests. The juice, however, possessed a much greater plasmolytic power than the precipitate solution. The degree of plasmolysis after 4 hours varied with the species tested, but did not correspond in any way with

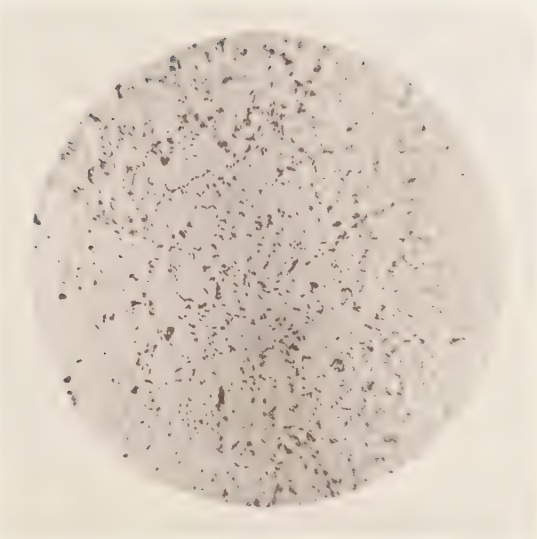

Fig. 1. Film from an emulsion of *B. solanisaprus* showing no agglutination by the potato precipitate solution after four hours.

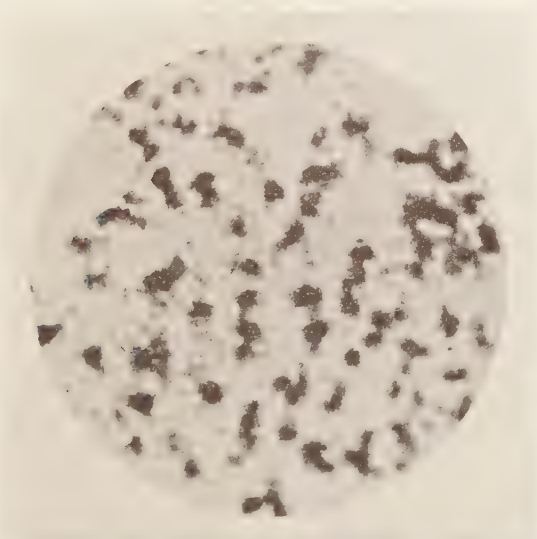

Fig. 2. Film from an emulsion of *B. fluorescens liquefaciens* showing almost complete agglutination of the bacteria by the potato precipitate solution at the end of four hours.

the degree of agglutination. The two potato parasites and *B. carotovorus*, which has been known to attack potatoes occasionally (Lacey (8)), remained almost unagglutinated and also unplasmolysed, but among the non-parasitic species, while the strongly agglutinated *B. fluorescens* and *B. pyocyaneus* resisted plasmolysis, in the *B. coli* and *B. vulgare* bacilli the cell contents were mostly reduced to granules within $\frac{1}{4}$ hour. The emulsions of sporing forms very quickly showed nothing but spores. Among the plant parasites, too, while *B. marginale* showed little plasmolysis of the agglutinated clumps after 4 hours, *B. delphinii* and *B. tumefaciens* were almost entirely plasmolysed. Agglutination therefore appears to be due to substances which can be precipitated from the juice by 95 per cent. alcohol, while this plasmolytic agent is evidently not precipitated to any great extent. The latter also differs from the agglutinating agent by being gradually lost or destroyed when the juice is exposed to the air; juice which had been left standing in the air for 24 hours and then filtered, exerted little plasmolysing effect on *B. vulgare* during 4 hours, and *B. coli* was only partially plasmolysed at the end of 2 hours; also after being boiled and kept for some time it showed practically no such power. This plasmolytic power of the juice must therefore be due to something in the fresh sap which is either oxidisable or volatile.

(3) In order to test whether H-ion concentration had any influence on these reactions, the potato precipitate solution was acidified, usually with weak acetic acid, or made alkaline with dilute ammonia or caustic soda. The *p*H values were determined colorimetrically by comparison with Clark's colour chart (3) in a parallel series of agglutination tubes directly after the test was set up, and frequently again at the end of 4 hours' incubation. The results with ammoniated solutions had often to be discarded owing to considerable change in reaction, and *B. coli*, in particular, showed a great tendency to neutralise an alkaline environment even in the space of 4 hours.

This third series of tests showed that in the presence of the particular constituents forming the precipitate there is for each species a certain H-ion concentration at which it suffers neither agglutination nor plasmolysis. For *B. solanisaprus* and *B. phytophthorus*, species which are pathogenic to potato, this value is *p*H 6·2, which is approximately the value of the H-ion concentration of the juice of the potato itself. With methyl red the reaction of the sap appeared to be *p*H 5·8–6·0; Wagner (15) determined it as 5·8 with lackmosol, while Youden and Denny (16), using slices of tissue immersed in various indicators, give the value as 6·1. This last value is probably nearest to the true one for the unoxidised

sap, for there is a slight but distinct rise of acidity on breaking up the tissue and thus exposing it to the air.

The complete list of *p*H values at which the 14 species tested are neither agglutinated nor plasmolysed by the potato precipitate is given in the first column of Table I.

Table I.

	Agglutination tests with potato precipitate		Cultural tests in Uschinsky solution	
	Bacteria unaffected *p*H	Bacteria plasmolysed *p*H	No growth *p*H	Growth *p*H
B. fluorescens liquefaciens ...	5·0	4·4	4·6	4·8
B. fluorescens non-liquefaciens ...	5·0	4·4	4·6	4·8
B. pyocyaneus	5·0	4·4	4·6	4·8
B. marginale	5·2	4·4	4·6	4·8 aggl.
B. tumefaciens	5·6	4·6	4·6	4·8
B. delphinii	6·0–6·4	5·2	5·2	5·5 slight
B. malvacearum	6·0–6·2	5·2	5·2	5·5
B. solanisaprus	6·2	4·6	4·6	5·2 slight
B. phytophthorus	6·2	5·2	5·2	5·5 slight
B. carotovorus	6·4–6·8	5·2	5·5	5·8
B. vulgare	7·2	5·8	6·4	6·9
B. coli	7·4	5·8	5·8	6·4 slight
B. dendroides	7·6	6·0	6·4	6·9 slight
B. mycoides	7·6–7·8	6·8	6·9	7·4 aggl.

For the plant parasites these points of non-agglutination all lie between *p*H 5·0 and 7·0; it is doubtful whether the *B. fluorescens* species ever become parasitic, but the closely related *B. pyocyaneus* can attack lettuce (Mehta and Berridge (11)). In the cases of *B. carotovorus* and *B. delphinii*, the points of non-agglutination were not well defined, but, for the former, which has been known to attack potato occasionally (Lacey (8)), it is slightly less acid than that of the true potato parasites.

For *B. coli*, as might be expected, a slight degree of alkalinity is favourable, while for *B. vulgare* the *p*H value lies near the neutral point. The two sporing organisms are hardly ever obtained in an emulsion of free bacteria, since they agglutinate spontaneously, but they grow most vigorously at about *p*H 7·6–7·8.

Each of these *p*H values may be called an "optimum" *p*H value for the species, since, judging by the case of the two potato parasites, it appears to be the degree of acidity most favourable for the attack of the species on the plant. It represents, however, the point at which the injurious effect of the sap on the invading organism is a minimum, rather than any true optimum condition of the bacterium.

On either side of this "optimum" *p*H concentration for any one species, change in either an acid or alkaline direction brings about agglutination. This increases with increasing change of *p*H, till plasmolysis sets in, due in this case to high H-ion or OH-ion concentration. This plasmolytic effect, which is not lost on exposure to the air, seems to be independent of that brought about by the fresh juice. Taking *B. coli* as an example, the "optimum" *p*H is 7·4, as determined by three independent tests. At *p*H 6·6 the emulsion is agglutinated after 4 hours, at 5·8 this agglutination is partially obscured by plasmolysis, and at *p*H 5·2 the bacterial contents are reduced to granules. Similarly agglutination increases with increasing alkalinity until plasmolysis sets in.

If the reactions described above are not due to unnatural laboratory conditions, or to the methods by which the precipitate is obtained, but do actually occur in nature, some light seems to be thrown on the susceptibility of the potato to certain bacterial species whose "optimum" *p*H has been shown to be that of the potato sap, and on its resistance to attack by forms whose presence in cuts and wounds must be of common occurrence.

Laurent (9), as long ago as 1899, found that the susceptibility of carrots and potatoes to disease could be varied by the use of lime or phosphatic manures, and confirmed his observations on the effect of lime in the field by showing that slices of potato and carrot soaked in weak alkali were attacked by *B. coli* and other saprophytic bacteria. Erwin Smith throws doubt upon his methods, but it seems quite possible that the effect of the alkali is to reduce the H-ion concentration of the sap to some value nearer the degree of acidity favourable to these organisms.

Cultural experiments.

In order to test whether the growth of various species in the presence of certain known constituents of the sap, *e.g.* phosphates and metallic salts, could be controlled in the same manner by varying the H-ion concentration, the growth of the fourteen types of bacteria was tested in Uschinsky's synthetic medium brought to the *p*H values 4·3, 4·6, 5·2, 5·5, 5·8, 6·4, 6·9, 7·4, since this medium contains besides asparagin, phosphates and small quantities of salts of di- and tri-valent metals.

Growth of the bacteria was slow, especially in the case of *B. delphinii* and *B. phytophthorus*, since the medium is poor in nitrogenous material. Turbidity usually only appeared on the second day, but by the fourth day the growth, if any, was well established; the tubes were kept for ten days at 20° C.

The results are summarised in columns 3 and 4 of Table I; column 3 gives the highest *p*H at which no growth occurred, and column 4 that at which growth was observed by the fourth day: the point at which inhibition of growth begins for each species lies between these two values. Column 2 is added for comparison; it gives the highest *p*H at which signs of plasmolysis were observed in the films made from the acidified potato precipitate solution. It will be seen that the maximum degree of acidity tolerated by each species in Uschinsky's solution lies near the *p*H at which acid plasmolysis of the organism was observed in the presence of the potato precipitate. The inhibition of growth in the Uschinsky cultures must be directly or indirectly due to the phosphates present, for omission of the metallic salts made no difference to the results. Plasmolytic effect on species with high "optimum" values, like *B. coli*, could moreover be observed in films made from agglutination tests with mixtures of K and Na acid phosphates in 0·1 per cent. solution. It seems probable therefore that the phosphates precipitable from the potato juice by the 95 per cent. alcohol, or rather the H-ion associated with them, is responsible for the plasmolysing and inhibiting action of the juice on those species whose optimum *p*H is higher than the *p*H of the sap itself, such as *B. coli*, *B. vulgare*, *B. mycoides* and *B. dendroides*. The immunity of the potato from attack by such organisms seems therefore to be dependent on the presence of phosphates in the sap; their action, however, may be reinforced by the plasmolytic constituent which is not precipitated by alcohol, but which is very active in the fresh juice.

The immunity of the potato from attack by species of bacteria with lower "optimum" *p*H values than 6·0 could not be attributed to the presence of phosphates in the sap. There was no inhibition of growth of these species in Uschinsky's solution at *p*H 6·2, 6·9, 7·4, which would correspond with the agglutinating effect of the potato precipitate on them at these values. Potassium and sodium phosphates, like other salts of these elements, have little agglutinating power on bacteria. If, however, traces of metallic salts such as manganese acetate or calcium chloride in a dilution of about 0·001 were added to the phosphate solutions, the films made after 4 hours' agglutination tests showed marked flocculation, especially with fluorescent types when the reaction of the solution was adjusted to *p*H 6·0–6·4 with NaOH; large patches of clustered bacteria were formed, especially in the manganese solutions, which were quite similar to those seen in films made from potato precipitate solutions (Fig. 2); *B. solanisaprus* and *B. carotovorus* on the other hand were hardly affected at all.

The agglutination due to the potato precipitate, whether it be due to traces of metallic salts or to other constituents, may not itself cause appreciable inhibition of growth, as agglutination is known to be a transient effect, but it may be a necessary preliminary to the action of some agent in the juice which is not precipitated by alcohol, for Mines (12) has shown that "neutral solutions of simple tri-valent ions quickly cause a marked alteration in the ionic permeability of a membrane." The more agglutinable forms like *B. fluorescens* may therefore be especially sensitive to bactericidal constituents of the potato sap not present in the precipitate.

On the other hand, possibly the mere fact that the mobility of the fluorescent forms is checked by agglutination during the period while incipient healing processes are taking place in the wound, may be sufficient to turn the scale in favour of the plant when invaded by this type of bacterium.

Summary.

1. The fresh juice of potato is shown to possess agglutinating action on certain bacteria, and strong plasmolytic power which is gradually lost on exposure to the air.

2. The agglutinating power is found to be due to substances which are precipitated from the potato juice by 95 per cent. alcohol, and is independent of any previous inoculation of the potato with bacteria.

3. At the natural H-ion concentration of the potato sap the precipitate solution agglutinates and plasmolyses non-pathogenic species to potato in varying degrees, but the pathogenic species tested were unaffected by it.

4. The agglutinating power of the potato precipitate for any one species varies with the H-ion concentration of the solution; each species remains unagglutinated and unplasmolysed at one definite H-ion concentration.

5. This point of non-agglutination at which the species appears to be least affected by the potato precipitate, was found to be approximately the H-ion concentration of the sap of the potato itself, *i.e.* about *p*H 6·2, in the case of the two species causing soft rot in the potato. The twelve species non-pathogenic to potato which were tested have non-agglutination points above or below this.

6. The precipitate has a plasmolytic as well as an agglutinating action on any species at *p*H values more acid than its point of non-agglutination; this has been shown by cultural experiments to be probably dependent on the presence of phosphates in the precipitate.

7. The agglutinating power may possibly be due to traces of salts of di- and tri-valent metals in the precipitate.

The writer wishes to record her indebtedness to Prof. V. H. Blackman and to Dr S. G. Paine for the laboratory facilities accorded her, and for helpful interest and criticism in the preparation of this paper. Especially her thanks are due to Dr Paine for the two photographs illustrating agglutination.

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THE MORPHOLOGY AND PHYSIOLOGY OF TWO LACTOSE-FERMENTING YEASTS AND CHEMICAL CHANGES DURING THE RIPENING OF CHEESE FROM MILK CONTAINING THEM

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(With 4 Text-figures.)

CONTENTS.

I. Introduction.

Two yeasts isolated from gassy cheese at the National Institute for Research in Dairying, Reading, were found to possess the power of fermenting lactose, and, since little is known of the chemical behaviour of lactose-fermenting yeasts or of the part they play in the ripening of cheese, this investigation was undertaken.

Search of the literature shows that several lactose-fermenting yeasts and torulae have, from time to time, been discovered in milk and milk products, probably the first being that of Grotenfelt (1889) described under the name of *Saccharomyces Lactis Acidi*, while others were added by Adametz (1889), Beijerinck (1889), Kayser (1891), Freudenreich and Jensen (1897), Jørgensen (1898), Harrison (1902), Jensen (1902), Mazé (1903), Dombrowski (1910), Hunter (1918) and Hammer and Cordes (1920). Dombrowski studied a variety of yeasts isolated from milk and other products, including Mazun, Yughourt, Kefir, cream and a milk starter. Of 26 cultures so obtained 10 fermented lactose and none fermented maltose. Of the lactose-fermenters four were *Saccharomyces* species, as evidenced by spore formation, and the remainder were *Torulae*. Hunter described a lactose-fermenting yeast responsible for foamy cream. The organism was oval in shape, averaging 5μ by 2μ in size, did not form spores and had an optimum temperature for growth at 37° C. or higher.

Dombrowski and Hunter review the literature cited above. There therefore seems no useful purpose to be served by extending a discussion of it here.

II. Description of the yeasts under investigation.

For the purposes of this description the yeasts will be designated as *B* and *C*.

Morphological characters. Preparations made from cultures some months old and stained with cold carbol fuchsin showed the cells to be mainly oval, with occasionally elongated and sausage-shaped forms. Subcultures from beerwort agar (48 hours) showed much less tendency to elongation. Budding was evident in both *B* and *C*. Measurement of the unstained cells from a 48-hour culture on beerwort agar gave the following dimensions (an average of 100 measurements in each case):

B	Average ...	...	4·5μ by 3·6μ
	Maximum	...	6·7μ by 5·1μ
	Minimum	...	2·9μ by 2·4μ
C	Average ...	...	5·0μ by 3·8μ
	Maximum	...	8·6μ by 5·2μ
	Minimum	...	2·9μ by 2·5μ

Ascospores were formed in *B* as the result of an isogamic fusion, usually resulting in the formation of four spores which were variously disposed in the two gametes. The shape of the ascus varied considerably

but was usually of a dumb-bell appearance. One or two cases were noted in which only two spores appeared. No spores were formed in *C*. It must therefore be recognised as distinct from *B* although in many respects it behaved similarly in culture (Fig. 1).

Cultural characters in various media. A pure culture of each yeast was first obtained by the dilution method (Paine, 1927). For a study of the cultural characters sugar broths and solid media were used. Incubation was at 30° C. unless otherwise stated. Sugar broths consisted of a

Fig. 1. Appearance of ascospores in *B*.

solution of peptone 1 per cent., common salt 0·25 per cent. and sugar 1 per cent., adjusted to a *p*H of 7·3 before sterilisation.

The schedule following describes briefly the appearance on the various media and the biochemical characters exhibited by the two yeasts. Since on many media the yeasts *B* and *C* gave identical growth the description has been arranged thus:

Group 1. Growth characteristics on media showing no distinction between the two yeasts.

Group 2. Growth characteristics on media showing noticeable differences between the two yeasts.

Group 1.

Solid media:

Gelatin stab. Growth slow, following the line of inoculation. Consisted of small, isolated, spherical colonies presenting the appearance of minute bubbles. Small, round, moist surface colonies. No liquefaction. Incubation at 20° C.

Gelatin plate. Small, round, white, wet-shining colonies. No liquefaction. Incubation at 20° C.

Gelatin streak. Very thin, dry, white, translucent growth. Had the appearance of a dry film on the gelatin. Incubation at 20° C.

Agar plate. White, wet-shining, raised colonies in 48 hours. Later colonies opaque with translucent edges. *C* was of noticeably slower growth than *B*. Slight fruity odour.

Agar streak. Thin, rather dry, yellowish-white growth in 24 hours. Growth moist but sparse after 3 days. Margin well defined though little growth in its vicinity.

Beerwort agar streak. Copious, greyish-white growth in 24 hours. Thick and continuous after three days, with a raised margin. After several days the margin had spread outwards in a smooth, slanting growth. In the case of *C* the margin was more serrated than in *B*. There was a strong fruity odour in both cases.

Separated milk agar plate. } The growth characteristics here were the same as
Separated milk agar streak. } for the corresponding plates on beerwort agar.

Sugars:

Glucose. Acid with white sediment but no gas after 24 hours. After 48 hours small bubbles of gas formed. After three days gas increased considerably. After four days gas decreased to small bubble. After six weeks still slightly acid but gas had disappeared; yellowish-white sediment.

Lactose. Acid and gas formed after 48 hours, in larger quantity than in the case of glucose. Gas disappeared after six weeks and liquid was neutral in reaction.

Sucrose. Acid after 24 hours. Acid with gas formation after three days. Gas disappeared and liquid was neutral in reaction after six weeks.

Galactose. Acid after 24 hours. Formation of gas after three days; white sediment. After six weeks gas disappeared, medium was slightly alkaline and rather turbid; yellowish-white sediment.

Levulose. Acid with white sediment after 24 hours. Formation of gas after 48 hours. After four weeks still acid with yellowish-white sediment. After six weeks gas had disappeared while the medium was slightly alkaline and rather turbid.

Maltose. Very slightly acid after 48 hours. No gas. After several days distinctly alkaline and rather turbid. After seven weeks condition unchanged.

α Methyl glucoside. No change after 24 hours. Slightly acid with slight sediment after 48 hours. No gas. After several days medium became alkaline. After six weeks still alkaline and rather turbid.

Raffinose. No change for some days. Medium then gradually turned alkaline with formation of slight white sediment. After six weeks still alkaline and rather turbid.

Various broths:

Mannite. Slightly acid after 24 hours. After four days still slightly acid; no gas. After six weeks still acid; medium turbid with yellowish-white sediment. After five weeks neutral. After seven weeks alkaline.

Dextrin. Slightly acid after 24 hours. After several days medium quite alkaline. After six weeks strongly alkaline. Yellowish-white sediment.

Peptone water. Slight white sediment after 24 hours. After six weeks fairly thick white sediment. Liquid clear; no pellicle; no ring.

Nitrate. Slightly alkaline after several weeks. Otherwise no change.

Uschinsky solution. Very slightly alkaline after several weeks. Otherwise unchanged.

Litmus milk. Slightly acid after 24 hours. After 48 hours evolved considerable quantities of gas on shaking. Noticeable odour of alcohol. No further change after several weeks. Milk not curdled.

Group 2.

Beerwort agar plate:

B. Silky white, round, wet-shining colonies in 48 hours. Moist-shining flattened colonies with dense centre after three days. After five days the centre was yellowish-brown with a white margin. After two weeks the colonies were quite large and the centre was more deeply tinted than the margin. Later the centre turned a chocolate colour and consisted of several concentric rings. A very pronounced pungent fruity odour was noticeable which gradually disappeared with age.

C. Minute, white, round, wet-shining colonies after 48 hours. After several days they became more raised than *B*, of a creamy colour, and marked in sectors. The same creamy colour was preserved for some weeks. The same pronounced fruity odour as in *B* was observed. The chocolate colour associated with *B* was a somewhat variable characteristic. Sometimes it was of quite a deep brown colour and at other times only a light brown on the same medium. The concentric rings in the colonies of *B* and the sectors in the case of *C* grew more pronounced with age and were most noticeable in the case of giant colonies incubated at a low temperature and kept for three or four months.

Beerwort:

B. Very slight white ring formed at the surface after 24 hours. After four days the ring was fairly thick and there was a white sediment and a fruity odour. Considerable gas was evolved on shaking. After two weeks the ring was quite thick with a slimy appearance.

C. No apparent change in 24 hours. After several days there was a thick white sediment and a fruity odour. Gas evolved on shaking. After two weeks there was usually no further change, but occasionally a thin white ring was observed.

In the case of *B* the ring was always observed, while in the case of *C* there was usually no ring at all.

III. Comparison of the colonies and rate of growth of *B* and *C* and their temperature relations.

A suspension of each yeast was made in sterile water and single point inoculations of both made on the surface of beerwort agar in the same Petri dish. Four dishes were prepared in this way and were incubated at 20, 25, 30 and 37° C. respectively. The colonies were examined and the relative rate of growth examined by measurement of their diameters. At 20° C. and 25° C. very little difference in the appearance of the colonies could be detected. Both *B* and *C* produced round, white, flat colonies with a dull, moist centre and a shiny translucent margin. At 30° C. differences were noticeable and have been recorded below. At 37° C. *B* grew very slowly and after several days produced a small round colony with a light yellow centre and a grey margin. *C* would not grow at 37° C. Incubation at this temperature therefore provided a means of distinction between the two yeasts.

The results of measurements at various temperatures are shown below:

		20° C.	25° C.	30° C.	37° C.
24 hours	*B*	No growth	1·5 mm.	3·0 mm.	No growth
	C	„	1·5	1·0	„
3 days	*B*	3·0 mm.	6·0	9·5	1·5 mm.
	C	2·5	6·0	6·5	No growth
7 days	*B*	5·0	8·0	13·0	2·5 mm.
	C	4·5	8·0	8·5	No growth

It will be seen that for the first 24 hours the optimum temperatures are 30° C. for *B* and 25° C. for *C*. After that the optimum temperature for both is 30° C. *C* was always of slower growth than *B* on all the media tested. Moreover *B* grew much more quickly at 30° C. than at 25° C. while *C* grew equally well at both temperatures.

Thermal death point. 48-hour cultures in beerwort were used and a period of 10 minutes taken as the standard of time required to cause

Fig. 2. Giant colony, *B*.

death. The thermal death point of *B* was found to be between 52° C. and 54° C. and of *C* between 50° C. and 52° C.

Composition of gas evolved during fermentation. Inoculations were made into Einhorn Saccharimeter tubes filled in one case with beerwort and in the other with 1 per cent. lactose broth. In each case the gas evolved by *B* and *C* was entirely absorbed by strong caustic soda. Carbon dioxide was therefore the only gas produced during fermentation.

Giant colonies. Single point inoculations of *B* and *C* were made on beerwort agar and incubated at 10–12° C. for 16 weeks. Growth was very slow and very little difference could be detected in the characters of the colonies for three or four weeks, but after two months the differences were well defined. Since these giant colonies provided a means of distinction between the two yeasts a detailed description of their appearance after four months is of interest:

Fig. 3. Giant colony, *C*.

B. Light brown colour except for $\frac{1}{4}$ in. at the edge which was of a creamy colour, and the extreme margin, which was grey and translucent. The centre was raised and of a dull "frosted" appearance to a diameter of about 1 in. Outside that the colony was smooth to the edge and consisted of four thin, concentric bands, surrounded by a system of radial lines, some broken and some entire, giving a crinkled appearance (Fig. 2).

C. Rather larger than *B* and comparatively smooth in appearance,

the centre not being raised. The central portion was of a slight yellowish-brown tint. The colony was divided into two sectors, one sector being well defined and of a somewhat different appearance from the remainder. It was smooth and consisted of thin, concentric bands from the centre to the edge. The remainder was of a shiny "frosted" appearance to within $\frac{1}{2}$ in. of the edge, where it was quite smooth with thin, concentric, wavy bands, ending in a grey, translucent, wavy margin. There were a few radial lines not reaching to the centre (Fig. 3).

IV. Comparison of yeasts *B* and *C* with other lactose-fermenting yeasts.

Two strains of lactose-fermenting yeasts were available for comparison and were obtained from the Collection of Type Cultures at the Lister Institute, London. One of these was *Saccharomyces fragilis* Jorgensen and the other a yeast which had been isolated and presented by Thaysen. Both proved to have many characteristics in common with *B* and *C* but were distinguished from *B* by the absence of sporulation, and from *C*, *S. fragilis* differed markedly in shape, the cells being more elongated and the prevailing form sausage-shaped. Thaysen's organism was considerably larger, averaging $6{\cdot}5\mu$ by $4{\cdot}8\mu$ and was characterised by an optimum temperature for growth in the neighbourhood of 45° C. The appearance of its giant colony was also markedly different.

Reference to the description given by Dombrowski (1910) of his new species *Zygosaccharomyces lactis* shows that yeast *B* agrees in many respects with this species, for instance in its copulation and spore formation, size of cells, sugar fermentations and absence of milk coagulation, so, although Dombrowski's description is a little brief, there seems every probability that yeast *B* belongs properly to this species. Yeast *C* is properly termed a Torula and its description has been rather fully given in this paper so that others who follow may know what class of organism the authors had before them. It in all probability is to be identified with one of the earlier discovered lactose-fermenting torulae; the descriptions of these, however, are too meagre to enable one to decide between them.

V. Action of the two lactose-fermenting yeasts on the protein and carbohydrate of milk.

The object of the first series of experiments was to discover whether the yeasts *B* and *C*, though they could not be classed as liquefiers of gelatin, had the power after a lengthy period of incubation to cause

a breakdown of the casein in milk. For this purpose fresh separated milk was used. Flasks containing 90 c.c. and tubes containing 10 c.c. were plugged and sterilised by intermittent steaming. The quantities of 10 c.c. were now inoculated, some with *B* and some with *C*, by transfer from a 48-hour culture on beerwort agar. These were incubated at 30° C. for four days to ensure a vigorous growth and were then poured under aseptic conditions into the flasks, the total volume in the latter being 100 c.c. In this way, by commencing with a sufficiently large and vigorous inoculum, good growth of the yeast in the comparatively large volume of milk (100 c.c.) was ensured. Cultures so prepared were incubated at 20° C. At intervals for a period of 16 weeks the extent of protein degradation in these cultures was measured by Sörensen's method of formol titration. The contents of a flask were well whisked to break up any deposited sediment and transferred quantitatively to a 250 c.c. graduated flask, the liquid being made up to the mark with distilled water. The contents were well shaken to ensure uniform distribution and an aliquot portion abstracted with a pipette. This was boiled for three minutes to expel CO_2 and titrated with decinormal sodium hydroxide, using phenol-phthalein as an indicator. Neutralised formalin was now added and the mixture again titrated to neutrality. The first reading then gave a measure of the acidity, and the difference between the first and second readings gave a measure of the peptide scission. Results were compared with those obtained for similar flasks of uninoculated milk kept under the same conditions.

The following tables show the effects observed. Table I represents the increase in acidity in terms of decinormal alkali, and Table II the increase in formol titration.

Table I.

Increase in acidity per 100 c.c. milk.

Time	c.c. of *N*/10 NaOH	
	B	*C*
1 week	3·36	3·46
2 weeks	18·55	13·93
4 „	17·14	17·44
9 „	17·85	18·05
13 „	17·32	—
15 „	15·19	14·97

Table II.

Increase in formol titration per 100 c.c. milk.

Time	c.c. of *N*/10 NaOH	
	B	*C*
1 week	0·37	1·06
2 weeks	0·19	1·55
4 „	0·57	2·22
9 „	5·23	7·95
13 „	9·84	—
15 „	12·64	17·13

It will be seen that, broadly speaking, the acidity increased until the end of the second week, or shortly after, and then remained constant for some weeks. After a rather lengthy period the acidity appeared to decrease. Since, as will be shown later, all the lactose was used up after about 14 to 17 days it is fairly safe to infer that the acid was a decomposition product of the lactose. Tests for lactic acid with Uffelman's solution failed to give positive results. For the first two weeks there

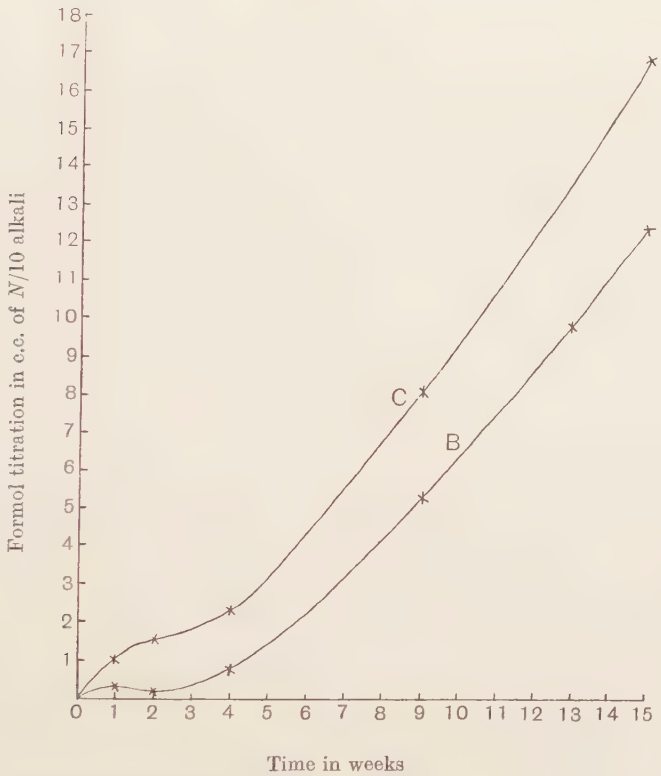

Fig. 4. Curves showing rate of protein degradation in milk by the yeasts *B* and *C*.

was certainly no appreciable increase in the formol titration and after four weeks the amount was very small, so that protein decomposition was not simultaneous with that of the lactose. After 9 weeks there was an appreciable peptide scission, and after 15 weeks a fairly considerable amount. From beginning to end *C* appeared to have a greater effect on the proteins than *B*, and at the end of the fifteenth week the difference was quite marked.

Similar experiments were made with milk inoculated with *Streptococcus lacticus* and with milk inoculated with a mixture of *Streptococcus lacticus* and the yeast *B*. In the former case inoculation was made direct into quantities of 90 c.c. of milk and the flasks were incubated at 37° C. for 18 hours before incubating for the period of the experiment at 20° C. In the case of the mixed culture the yeast was first inoculated into 10 c.c. of sterile milk and incubated for three days at 30° C. The *Streptococcus lacticus* was meanwhile inoculated into the 90 c.c. of milk in the flasks, and after 18 hours' incubation at 37° C. the culture of *B* was poured in under aseptic conditions and the flasks incubated at 20° C. In this way some attempt at comparable conditions was made, for a slow-growing yeast could not well compete with a fast-growing *Streptococcus* unless first made into a vigorous inoculum.

The results of these experiments showed that at the end of 10 weeks no protein degradation had occurred in either series. Moreover at the end of that period there was still a considerable quantity of lactose remaining in both sets of cultures.

VI. Effect on the lactose.

A series of experiments was next made to discover the relative rate of decomposition of the lactose in milk of (*a*) the yeast *B* alone, (*b*) the *Streptococcus* alone, and (*c*) a mixture of the yeast and *Streptococcus lacticus*. For this purpose cultures in flasks were prepared as in former experiments, one set being inoculated with a 48-hour culture of the yeast, a second set inoculated with *Streptococcus lacticus* and incubated at 37° C. for 18 hours before incubating at 20° C., and a third set treated in exactly the same way as the second set and then inoculated with 10 c.c. of a 48-hour culture of *B* before incubating at 20° C. Lactose determinations were made on the three sets of cultures at frequent intervals for 16 days and then at less frequent intervals. The contents of a flask were well whisked as before and made up to a volume of 250 c.c. It was found that a satisfactory homogeneous emulsion was obtained by vigorous shaking and an aliquot portion easily abstracted for analysis. The protein and fat were precipitated with acid mercuric nitrate, filtered and thoroughly washed with warm water, and the lactose was determined in the filtrate by Bertrand's method.

The results of the experiments are shown in Tables III and IV where the first column in each represents the time after inoculation, the second column gives the percentage of lactose present in the milk

at that time, and the third column shows the percentage of the original lactose which had been decomposed at the corresponding time.

Table III.

Yeast B.

Time	% lactose present	% decomp.
0	4·73	0·00
30 hours	4·46	5·71
78 „	4·12	12·90
128 „	3·35	28·33
168 „	2·99	36·79
11 days	0·99	79·16
14 „	0·69	85·41
17 „	Trace	100·00
—	—	—

Table IV.

Yeast + Strep. lact.

Time	% lactose present	% decomp.
0	4·17	0·00
—	—	—
78 hours	3·87	7·19
128 „	3·76	12·23
168 „	3·51	15·83
10 days	3·33	20·14
14 „	3·28	21·34
8 weeks	1·47	62·02
10 „	0·28	93·28

In the experiments on the cultures of *Streptococcus lacticus* alone it was found that in the early stages of growth the lactose content varied so much from flask to flask that comparable results were impossible. In relation to the effects of the yeast and of the yeast plus *Streptococcus lacticus*, however, the rate of consumption of lactose in the cultures of the *Streptococcus* alone was, after an initial rapid growth, very slow. Thus after eight weeks only 14·01 per cent. of the lactose had been used up and the amount was increasing very slowly indeed.

It will be seen from these results that the yeast alone uses up the lactose far more quickly than the *Streptococcus* or a mixture of the yeast and the *Streptococcus*. The rate of consumption of the lactose for a mixture of the two may be said to be a mean of the rates for either of the two singly. This may indicate either that the *Streptococcus* considerably retards the growth of the yeast or that the yeast feeds on the decomposition products of the lactose which are provided by the *Streptococcus*. The latter idea pointed the way to an experiment to determine whether either of the yeasts could utilise lactic acid as a food. For this purpose a medium containing 0·3 per cent. meat extract, 0·25 per cent. NaCl, 1·0 per cent. peptone and 1 per cent. lactic acid was prepared, transferred in quantities of 10 c.c. to tubes and sterilised in the autoclave at 115° C. for 20 minutes. Some tubes were then inoculated with yeast *B*, some with yeast *C* and some left as control, the whole being incubated at 30° C. After several days the cultures of *B* and *C* showed turbidity and a slight white sediment indicating growth. The lactic acid present was determined by boiling the liquid for three minutes to expel CO_2 and

titrating to phenol-phthalein with decinormal sodium hydroxide. Results are shown in the following table:

Time	c.c. of $N/10$ NaOH	
	B	*C*
0	10·11	10·11
7 days	9·54	9·65
17 „	8·91	8·90

These indicate a small but definite decrease in titratable acidity which may reasonably be assumed to show a decrease in the lactic acid content. *B* and *C* would appear then to be able to utilise lactic acid as a food.

VII. Effect on cheese made from milk inoculated with yeasts *B* and *C*.

Two sets of four 10 lb. cheeses (English Cheddar) were made, the cheeses in each set being from the same milk. In one set two cheeses were inoculated with yeast *B* and two left as control. In the other set two were inoculated with yeast *C* and two left as control. Inoculation was made by adding to the vat two test-tubes full of a three-day culture of the yeast incubated at 30° C., and leaving overnight until the morning's milk was added. The cheeses were examined from time to time throughout the course of ripening and the inoculated ones compared with the control. After the first week the inoculated cheeses exhibited a "blown" appearance due to the evolution of CO_2. Samples were taken by boring from the outside to the centre with a circular borer. Those from the inoculated cheeses were of a noticeably darker appearance, with a fruity odour which became more pronounced on mashing with warm water. The flavour was bitter and very yeast-like and unpleasant, while the texture was more open than the control cheeses and in consequence a portion was found to be more friable on grinding with sand in a mortar. The fruity odour disappeared after about three weeks, and the "blown" appearance of the cheeses was less and less noticeable until, at the end of four months, they had a normal appearance.

Attempts were made throughout the course of ripening to determine whether there was any distinct difference in the extent of protein degradation. For this purpose about two grams of cheese, taken from a sample boring, were well ground with fine sand, made into a suspension with water and kept at a temperature of 55° C. for half an hour. Formol titrations were then made in the same way as described for the milk. It was found, however, that results varied so much in the same cheese,

according as the samples were taken from the inner or outer portions, that comparable results between two cheeses were impossible. After five months the cheeses were cut and examined. The colour of the inoculated and control cheeses was practically the same, but the texture of the former was more open and the flavour was distinctly different. The unpleasantness of the inoculated ones had disappeared, but there was still a slight fruity flavour although the bitterness was absent.

VIII. Chemical comparison of two ripe cheeses[1].

A chemical comparison of two cheeses, one inoculated and one control, was made after eight months' ripening. For this purpose two whole cheeses, one control and one inoculated with yeast *C*, were sampled by grinding about a kilogram of each on a cheese grater and thoroughly mixing to ensure representative sampling. The moisture, fat and nitrogen contents were then determined in duplicate in the case of each cheese. For the moisture determination about five grams of the ground cheese were weighed and dried in a vacuum desiccator for four days. At the end of that time the remaining moisture was driven off in the steam oven, the process taking 24 hours. Fat was determined by extraction with ether by Soxhlet's method. Nitrogen was determined by Kjeldahl's method on about a gram of the ground cheese. For investigation of the decomposition products of the proteins the fat-extracted material was used. For this purpose about 50 grams of the ground cheese were fat-extracted for five hours and the ether and moisture remaining were driven off by placing in an air oven at 55° C. for 24 hours. At the end of this time the product was hard and perfectly friable. It was therefore ground as finely as possible by passing through a mill and then rubbed up in a mortar. A suspension of the powder so obtained was made by well mixing with sterile distilled water, transferring to a sterile 500 c.c. flask and making up to the mark with sterile water. Sterile water and flask were used in order to minimise any protein decomposition due to bacterial contamination. The suspension was well shaken to ensure uniformity and two portions of 25 c.c. quickly pipetted off for nitrogen determination. The remainder of the suspension was left to stand for 16 hours in order to allow all soluble substances to pass into solution.

At the end of this time the suspension was again well shaken and two further portions abstracted for formol titration. The remainder of the suspension was filtered through coarse filter paper, the filtrate being of

[1] This work was done by one of us (L.A.A.) at the National Institute for Research in Dairying, Shinfield.

a clear colour. A nitrogen determination and formol titration were made on portions of 25 c.c. of the filtrate. 50 c.c. of the filtrate were run into 200 c.c. of neutralised absolute alcohol and the mixture made up to 250 c.c. by the addition of neutralised 80 per cent. alcohol. This was left for 24 hours and then filtered. A nitrogen-content determination and formol titration were made on aliquot portions of this filtrate.

In this way three sets of figures were obtained, viz. the nitrogen-content and formol titration for (1) the aqueous suspension, (2) the aqueous filtrate, (3) the alcoholic filtrate. In the case of the suspension all the decomposition products of the casein, from the protein itself to amino acids, would be present. In the aqueous filtrate casein and some of the higher decomposition products would be eliminated. The object of the 80 per cent. alcohol was to provide some means of partial separation of the amino acids and the lower polypeptides from the higher polypeptides and peptones. The alcoholic filtrate therefore contained the lowest decomposition products of the casein. For the formol titration on the suspension and aqueous filtrate Foreman's method as modified by Harris (1923) was used, phenol-phthalein and alcohol plus formalin being preferred to thymol-phthalein and alcohol alone, as giving a better end-point. This determination also gave a measure of the acidity of the liquid as well as of the extent of peptide scission. The nitrogen-content of the alcoholic filtrate was determined by means of a micro-Kjeldahl apparatus. 5 c.c. of the filtrate were evaporated (in hard glass incineration tubes on the water bath) with five or six drops of concentrated hydrochloric acid to a point just short of dryness, so as to eliminate all the alcohol, while at the same time leaving behind all the volatile bases. To the residue 1·5 c.c. of H_2SO_4, two drops of $CuSO_4$ solution and a little K_2SO_4 were added. This was incinerated and distilled from alkali into $N/70$ H_2SO_4 in the usual way. An iodimetric titration was used to determine the quantity of $N/70$ acid still remaining after distillation. For this purpose 5 c.c. of a 1 per cent. solution of KI, 1 c.c. of a 1 per cent. solution of KIO_3, and 2 c.c. of a 1 per cent. solution of soluble starch (saturated with NaCl) were added to the distillate. After leaving for five minutes to allow complete separation of the iodine the mixture was titrated with $N/70$ $Na_2S_2O_3$.

Results of analysis.

	Control cheese	Inoculated cheese
Moisture content ...	21·68 %	29·85 %
Nitrogen content ...	6·12	5·55
Fat content ...	39·44	33·44

Protein degradation—Nitrogen distribution.

	Control cheese	Inoculated cheese
Aqueous suspension ...	1·0000 gm.	1·0000 gm.
Aqueous filtrate ...	0·3915	0·4537
Alcoholic filtrate ...	0·2187	0·2680

Acidity and formol titrations (per gram of nitrogen present in each case).

		Control cheese	Inoculated cheese
Aqueous suspension	Acidity	81·48 c.c.	72·27 c.c.
	Formol titration	75·31	138·54
Aqueous filtrate	Acidity	46·52	45·07
	Formol titration	70·64	109·90
Alcoholic filtrate	Acidity	134·6	138·5
	Formol titration	269·6	396·4

Results of acidity and formol titrations are expressed in each case as c.c. of $N/10$ sodium hydroxide required for neutralisation. The figures given are calculated per gram of nitrogen present in each case and therefore afford a basis for strict comparison.

IX. Discussion of results.

These results show a definite difference between the two cheeses in the major analysis, in the acidity and in the protein decomposition products. The apparent differences in the fat and nitrogen-contents are explained of course by the difference in the moisture-content. This would seem to indicate a difference in the texture of the cheeses giving rise to a different degree of tenacity for the moisture originally present. The results of the acidity titrations on the aqueous suspensions show that the inoculated cheese is considerably less acid than the control cheese, a result which may be expected both on general grounds and from the experiments already conducted on milk, inoculated in one case with the yeast alone and in the other case with the yeast and *Streptococcus lacticus*. Thus since the yeast gives rise to an alcoholic fermentation with the production of very little acid it may be expected to inhibit to a corresponding extent the formation of lactic acid by the starter introduced into the cheese at the commencement. This is borne out by the experiments on milk where it was shown that the yeast uses up the lactose more quickly than does the *Streptococcus* alone. Moreover, there was some indication that the yeast is capable of utilising the lactic acid as a food and thereby decreasing the acidity.

The formol titrations are perhaps the most interesting results since they give an indication of the extent of protein degradation. Those for the aqueous suspension indicate a greater amount of general decomposition in the case of the inoculated cheese. Those for the aqueous filtrate and

the alcoholic filtrate, combined with the figures for the nitrogen distribution, show both that the lower degradation products are present in greater amount and that they are of a lower order of degradation in the case of the inoculated cheese. Thus both the nitrogen-content and the formol titration *per gram of nitrogen present* are considerably greater in each case for the inoculated cheese.

The general conclusion is that the presence of a lactose-fermenting yeast in a cheddar cheese produces a more open texture, decreases its acidity, produces esters which impart a distinctive flavour, and brings about a greater decomposition of the casein.

X. Summary.

Two lactose-fermenting yeasts isolated from cheese have been described and though markedly similar in many respects they have been referred to separate species, the one being a Torula whose close relationship could not be definitely established, and the other being a true yeast believed to be correctly identified with *Zygosaccharomyces lactis* (Dombrowski).

The two yeasts have been found to have an appreciable proteolytic effect on the casein of milk after several weeks. The effect of one of the yeasts alone and of the yeast plus *Streptococcus lacticus* on the lactose of milk has also been studied.

Cheddar cheese inoculated with the yeasts has been found to differ considerably in quality and texture from the control cheese. A comparative study of the extent of protein degradation in two ripe cheeses, one inoculated with the Torula and one control, has shown the proteolytic effect of the yeast in the course of ripening.

The authors wish to express their thanks to Dr S. G. Paine for his constant interest and help in this work, and to Mr A. T. R. Mattick for many valuable suggestions.

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INVESTIGATIONS ON *HETERODERA SCHACHTII*, SCHMIDT. IN LANCASHIRE AND CHESHIRE

PART III. CERTAIN CORRELATIONS BETWEEN CROP YIELDS AND DEGREE OF INFESTATION

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(With 1 Text-figure.)

In a previous publication (2) the technique of estimating the degree of infestation of *H. schachtii* in the field was examined and an attempt made to correlate significant differences with the intensity of "eelworm disease" as estimated by visual observation. Although the results indicated that in those areas where disease had been noted comparatively recently there was a positive association between cyst count and intensity of disease, it was felt that a more accurate computation of the latter, by measuring crop yield, was necessary before any definite conclusion might be drawn. Furthermore, the fact that, in areas where disease had been observed more than three or four years previously, the cyst counts did not bear a close relationship to the state of the crop, indicated that *H. schachtii* was at most only one factor in the causation of what is termed "eelworm disease" of potatoes. It was decided, therefore, to lay down a number of plots on affected ground, make observations throughout the growing season, weigh the produce and compare the yields with the original and final degree of infestation as measured by cyst counts.

Experimental.

Field. Four series of plots, varying in size from 1/130 to 1/40 of an acre were laid down in different localities. The first three series were on peat and the fourth on peaty sand. The soil characteristics and farm practice, together with the notes on the first appearance of disease, have

already been fully described (2). The plots were sampled about the middle of January, 1928, and measurements made of cyst content and a number of chemical properties. About the middle of March, 16 of the 29 plots were treated with varying amounts of calcium carbonate to provide data for another object in view. All the plots received the same manurial dressing in May, prior to planting with potatoes. The variety employed was Great Scot and all the seed was taken from one consignment. Field notes were made throughout the growing season and the crops were harvested at the beginning of September, the weights being taken to the nearest pound. The plots were re-sampled in December and the same measurements made as on those samples of soil which were taken nearly twelve months previously.

Laboratory. The soil samples were allowed to reach an air-dry condition and then passed through a 2 mm. sieve. Cyst counts were made as described (*loc. cit.* p. 326) on the fine earth portion. For each sample determinations were made of (*a*) the *p*H electrometrically (3), (*b*) the "lime-requirement" by the Hutchinson and MacLennan method, and (*c*) the content of free carbonate by means of a Collin's calcimeter. The results have been collected in Table I.

Results.

Field observations. So far as could be gathered from observations made in the field, growth commenced quite normally, and little or no difference could be noted on the different plots. There was, however, a considerable number of misses associated directly with the fungus *Rhizoctonia solani* Kühn. By the middle of August it was quite obvious that all the crops were far below the average and that, in the series 1 and 3, they were, from the practical point of view, almost complete failures. In those cases the foliage had an unhealthy appearance and was so dwarfed that the drills were not completely covered. Series 2 was not quite so bad, but many poor patches were to be seen at irregular intervals. Series 4 was undoubtedly the best, but even there the crop was not up to standard.

Laboratory measurements. In Table I the limed and unlimed plots have been kept separate in order to facilitate comparison. Figures for the total yield of potatoes, including ware and chats, are given alongside the original, final and change in cyst count. The difference between the amount of calcium carbonate added and the original "lime-requirement" was closely associated with the final content of carbonate in the soil: since, however, the unlimed plots almost invariably showed an increase

Table I.

Results for series of plots.

Unlimed.						Limed.					
	Cyst count per 10 c.c. soil						Cyst count per 10 c.c. soil				
No.	Before	After	Change	Yield cwt./A	% $CaCO_3$ Change	No.	Before	After	Change	Yield cwt./A	% $CaCO_3$ Change
1 AB	11·8	11·1	−0·7	36	Nil	1 A	12·5	11·7	−0·8	40	2·75
1 BC	14·1	12·7	−1·4	23	0·15	1 B	10·8	13·0	+2·2	30	1·36
1 CD	17·8	12·5	−5·3	26	0·18	1 C	18·8	9·8	−9·0	19	1·99
1 DE	11·5	14·9	+3·4	39	Nil	1 D	14·7	15·0	+0·3	30	1·22
2 AB	20·8	22·7	+1·9	76	0·10	2 A	21·7	26·8	+5·1	114	0·35
2 BC	17·8	27·4	+9·6	94	0·29	2 B	19·7	25·6	+5·9	60	2·48
2 CD	10·6	15·5	+4·9	57	0·05	2 C	16·0	20·3	+4·3	53	0·93
						2 D	6·6	12·1	+5·5	105	0·87
3 A 1	21·0	13·3	−7·7	26	0·76	3 A 2	21·0	11·5	−9·5	34	1·52
3 B 2	25·0	21·8	−3·2	14	0·04	3 B 1	25·0	19·9	−5·1	28	1·24
4 A 3	17·0	20·7	+3·7	134	0·04	4 A 1	17·0	16·4	−0·6	122	0·72
						4 B 1	14·8	19·5	+4·7	119	0·28
4 C 1	19·7	23·9	+4·2	117	0·01	4 C 2	19·7	18·4	−1·3	91	0·14
4 D 3	18·6	19·4	+0·8	86	0·11	4 D 2	18·6	17·7	−0·9	87	0·30
4 E 2	9·0	12·8	+3·8	81	0·07	4 E 3	9·0	11·9	+2·9	108	0·25
						4 B 2	14·8	18·2	+3·4	99	1·26

in content of carbonate, owing, presumably, to the accidental transfer of lime from the adjacent limed plots, the increase in percentage of calcium carbonate has been given throughout. In view of the heavy dressings of lime and the comparatively short space of time elapsing since their application, not much stress could be laid upon the final *p*H figures for the limed plots, whilst the *p*H of the unlimed plots did not alter materially. Consequently, the *p*H values have not been included.

The most striking feature of the results is the very low yields obtained from the diseased areas upon which the plots were laid. A normal yield fluctuates about 10 tons per acre, but on those plots the yields vary from less than 1 ton to about 6 or 7 tons per acre; the average yield is little more than 30 per cent. of the normal.

As a preliminary step towards the elucidation of any associations, scatter diagrams were prepared from the various pairs of values. It was evident that no close association existed between the yield and the amount of calcium carbonate present or between the yield and the original cyst concentration of the soil. There was an apparent negative association of values representing change in cyst count and original cyst count, and the same thing could be said to a less degree of the change in cyst count and the increase in calcium carbonate. The most obvious association, however, lay between yield of potatoes and change in cyst

count. An examination of the data shows that, with few exceptions, the cyst count has increased when the yield is more than 50 cwt. and decreased when the yield is less than 50 cwt. The figures for yield and change in cyst count are plotted in Fig. 1, and the general trend of the results is fairly obvious.

In order to examine the possible associations more closely, "total correlation" coefficients for six pairs of values have been calculated and are shown in Table II.

Table II.

Coefficients of correlation between pairs of values.

	A	*O*	*L*
Y	+0·592	−0·131	−0·315
A	—	−0·409	−0·232
O	—	—	+0·044

The letters *A*, *O*, *L*, *Y* designate, respectively, alteration in cyst count, original cyst count, increase in percentage of calcium carbonate and yield. The correlation between *A* and *Y* has been calculated from the formula (1),

$$r_{AY} = \frac{\Sigma ay}{n\sigma_a\sigma_y},$$

where $\Sigma ay/n$ = the mean product of the deviations of *A* and *Y* from their means,

σ_a = the standard deviation of *A*,

σ_y = the standard deviation of *Y*.

For 29 sets of observations, the only correlation which is definitely significant is that between *A* and *Y*. The coefficient $r_{AO} = -0{\cdot}409$ has a probability between 0·05 and 0·02 and is, therefore, just significant.

The correlations between *A* and *Y* when *O* and *L* are eliminated are

$$r_{AY.O} = 0{\cdot}596 \quad \text{and} \quad r_{AY.L} = 0{\cdot}562,$$

which show that the association between *A* and *Y* is independent of the original cyst concentration, and is reduced slightly by the elimination of *L*, the increase in calcium carbonate present. It is not possible to say whether *A* and *Y* vary independently or the extent to which they may be interdependent, but certain factors apparently affect *A* and *Y* similarly and to an important extent compared with other factors, so that, generally speaking, over the diseased areas investigated the cyst concentration increases in proportion to the yield when that is greater than about 3 tons per acre, and decreases with yield when that is less than about 3 tons per acre.

The regression lines (*a*) and (*b*), represented by the equations

$$A = 0{\cdot}0737\,Y - 4{\cdot}215 \qquad \ldots(a),$$

$$Y = 4{\cdot}75\,A + 63{\cdot}73 \qquad \ldots(b),$$

have been inserted in Fig. 1. Those equations have been obtained from the coefficients of regression $b_1 = r\sigma_a/\sigma_y$ and $b_2 = r\sigma_y/\sigma_a$, from which $a = 0{\cdot}0737\,y$ and $y = 4{\cdot}75\,a$. The mean values for A and Y are respectively 0·73 and 67·2, so that $a = (A - 0{\cdot}73)$ and $y = (Y - 67{\cdot}2)$.

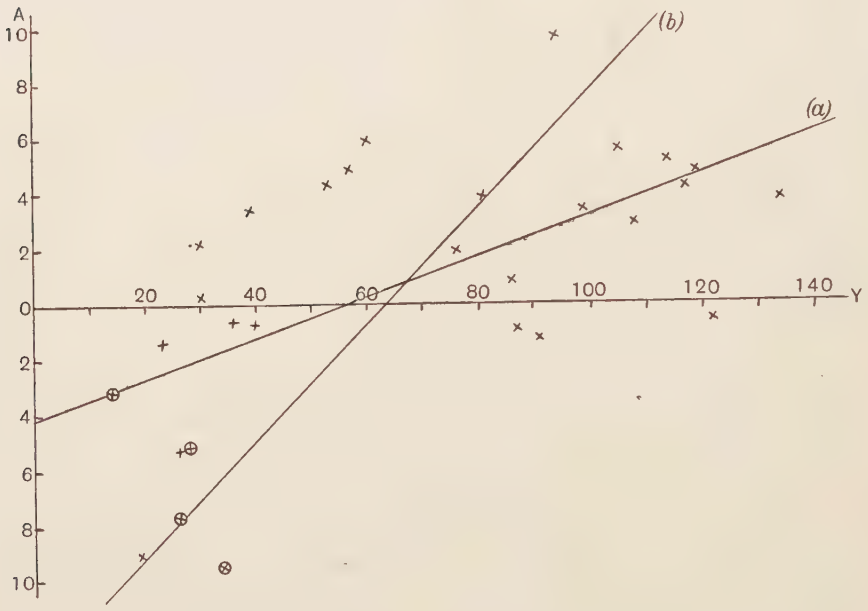

Fig. 1. Diagram showing association between yield (Y) and change in cyst content (A).

It is evident that the correlation depends upon a number of low values for Y, four of which are from series 3 and have been encircled. On account of the limitations of the experiments, a close analysis of the plot variation cannot be made, but it is possible that the association between A and Y may exist in the variations between plots and be bound up with the unknown factor fertility.

The coefficient of correlation $r_{AO} = -0{\cdot}409$ is not affected much by eliminating L and Y because $r_{AO.L} = -0{\cdot}411$ and $r_{AO.Y} = -0{\cdot}414$. The data, however, do not reveal whether the association between the original concentration and change in concentration of cysts is due to a causal relationship or whether both are affected by another factor. It

has been shown (3), for the soils under consideration, that no apparent association exists between cyst count and physical environment. The observed correlation between A and O simply indicates that, under the conditions of the investigation, the number of cysts tends to increase on cropping when the original concentration is less than about 17 and *vice versa*. It must be noted, however, that without the four large negative values for A from series 3 the observed correlation would become negligible.

Summary.

1. Data concerning infestation of *H. schachtii*, certain soil properties and crop yield, from a series of plots at four centres affected with disease, have been collected and examined.

2. Limitations imposed on the design of the plots have prevented a complete analysis of the variates, but the results obtained lead to the conclusion that eelworm infestation is not of primary importance in determining the yield of potatoes and that the cysts tend to increase in number only on a crop which is not a failure but which has been adversely influenced by some other factor.

The authors would like to express their thanks to Messrs I. S. Macdonald, J. Orr, E. Holmes Smith and I. Thomas of the Advisory Department at Manchester for their willing co-operation and assistance, and to Mr L. H. C. Tippett for his helpful criticism of the statistical treatment of the results.

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POLLINATION OF HARDY FRUITS: INSECT VISITORS TO FRUIT BLOSSOMS

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(With 1 Text-figure.)

CONTENTS.

1. Introduction.

In July 1926 the writer published a paper in the *Royal Horticultural Society's Journal* (28) which dealt with the insect visitors to fruit blossoms. That paper was necessarily a popular account on the subject; now a more detailed account of the work as carried out at Wisley during the years 1920–25 is given. The greater number of observations were carried out in the fruit plantations of the R.H.S. Gardens at Wisley, where many hours were spent during the blossoming periods of the various fruits for the purpose of studying the relationship between anthophilous insects and fruit pollination.

The problems were suggested by the Director of Wisley who put forward a plea in 1914 (5) to get the matter of the agents concerned in fruit pollination cleared up and suggested that a productive piece of

work would be done if the investigation were taken up seriously. The problems that were set were: firstly, to ascertain the species of insects concerned and, secondly, a study of the habits of the various insects which are found to visit the flowers of hardy fruit.

Whereas previous work in this country on the question of pollinating agents has been confined chiefly to lists of insects found visiting the blossoms (14, 15, 16), detailed observations of their habits have been few and scanty. The mere record of captures at the flowers is of relatively little value, for that takes no note of the number of visits an individual insect may pay.

Acknowledgments. I wish to express my sincere thanks to Mr F. J. Chittenden, Director of the R.H.S. Gardens at Wisley, for suggesting the research in 1920 and for reading through this paper and for help and kindly criticism during its progress. Grateful acknowledgment is made of the help received from Mr H. Britten (University of Manchester) and the late Mr E. B. Nevinson (Cobham) in identifying many of the Hymenoptera-Aculeata, and to Mr F. W. Edwards (British Museum, South Kensington) for great help in the identification of the Diptera, and to Mr W. D. Cartwright (Wisley) for allowing me access to the Meteorological Records which are taken daily by him.

2. Description and plan of orchards in which investigations were conducted.

The plantations at Wisley are favoured by reason of their close proximity to open country and pasture land, the district and position of orchards governing to a large extent the numbers of wild insects. There are no grass orchards at Wisley, the land beneath the trees and bushes of all kinds of fruit being cultivated.

The numbers of plants and varieties together with the area which each occupy have been tabulated (Table I), and position of the various fruits are shown (Fig. 1).

Hutson (17) remarks that the number of insects other than the hive bee acting as fruit pollenisers in southern New Jersey is small. This is not true of the Wisley plantations, especially in the spring of 1920 when not one hive bee was found on the blossoms of any fruit tree due to the absence of hives within a radius of two miles, and yet an excellent set of fruit was obtained through the agency of wild insects, particularly humble and wild bees.

Table I.

Type, number and varieties of hardy fruits on which the greater number of observations on their pollinating agents were made.

Plan figure	Fruit	No. of plants	No. of varieties	Area
1	Almond	2 standards	1	"Sevenacres"
2	Apple	390 bush	189	$1\frac{1}{8}$ acre
2 C	Apple	65 cordons	6	130 ft. run
3	Apricot	2 espaliers	1	South wall
*	Cherry	20 half-standards	4	250 sq. yards
4	Cherry	5 espaliers	3	North wall
5	Medlar	1 standard	1	Wild garden
6	Nectarine	5 espaliers	5	South wall
6	Peach	5 espaliers	5	South wall
7	Pear	234 bush	121	$\frac{7}{8}$ acre
7 C	Pear	80 cordons	6	160 ft. run
8	Plum	112 bush	56	$\frac{1}{2}$ acre
9	Quince	10 bush	4	120 ft. run
10	Blackberry	30	12	320 sq. yards
11	Loganberry	40	1	
12	Raspberry	900	32	$\frac{1}{3}$ acre
13	Strawberry	540	60	160 sq. yards
14	Black currant	51	8	150 sq. yards
15	Red and white currants	114	6 2	330 sq. yards
16	Gooseberry	174	72	430 sq. yards
17	Mulberry	1 standard	1	Wild garden
18	Nuts, cob and filbert	45	2	150 yards run
19	Chestnut, sweet	14	1	Wisley Common
20	Walnut	1 standard	1	"Sevenacres"

* Not shown on plan.

3. Factors influencing fruitfulness in orchards.

No amount of care to cultural details can induce fruitfulness in a large orchard unless pollen-carrying agents are present in sufficient numbers to ensure adequate cross pollination of the flowers. The lack of pollinating agents may be only one factor influencing fruitfulness in orchards, yet it is too often considered to be a negligible quantity, for less attention has been paid to the essential work of anthophilous insects in the pollination of entomophilous flowers than to any of the undermentioned factors.

The principal factors governing fruitfulness in orchards are:

(1) Lack of vigour—due to unhealthy root action caused by inefficient drainage and absence of essential soil nutrients or may occur in interplanted bush fruit through dense shade conditions.

(2) Errors in cultivation—a common error is the repeated application of nitrogenous manures which causes an over-production of wood and the failure of the plant to form fruit buds.

(3) Shy-bearing varieties and self-sterile varieties—the massing of self-sterile and inter-sterile varieties and the lack of foresight shown when planting these so that their flowering periods do not overlap.

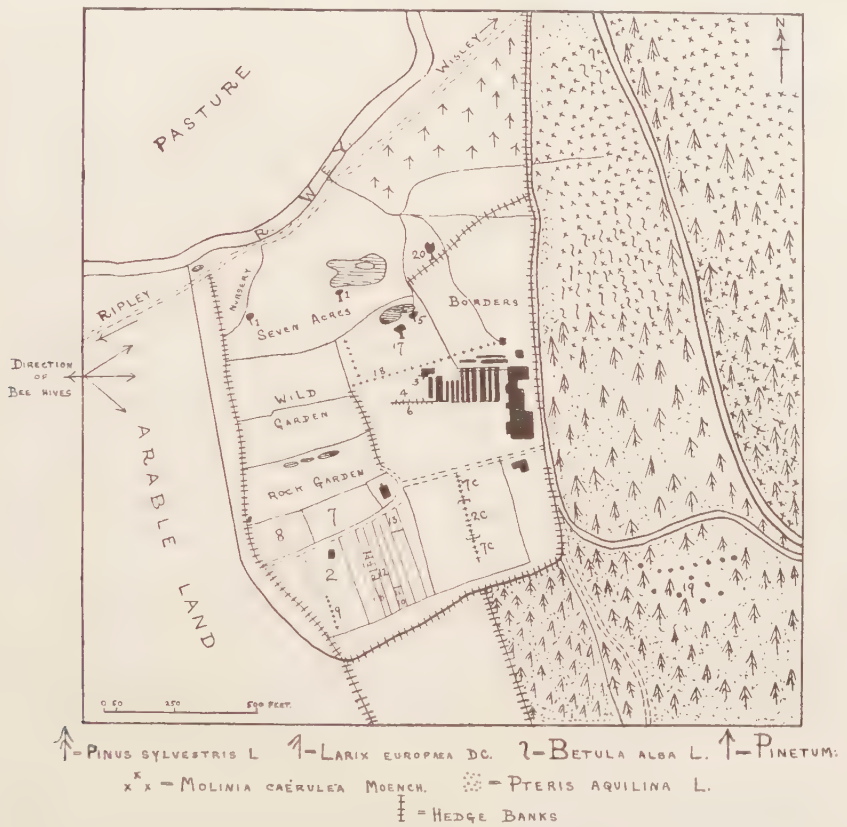

Fig. 1. Map of the Wisley Gardens and surrounding district, showing position of the various fruits on which observations were made.

(4) Unfavourable climatic conditions—drying winds injure the receptive stigmas; rain prevents pollen dissemination by closing the anthers or by preventing them from opening (Dorsey(7)); gales and hail showers too often remove the petals and so decrease the attractiveness of rosaceous flowers; low temperatures prevent the setting of fruit,

whilst frost, especially in low-lying regions, kills the stigma and retards the growth of the pollen tube. The indirect effect of unfavourable weather conditions at the time of blossoming is to reduce the number of pollinating agents, particularly domesticated bees.

4. Anemophilous and entomophilous flowers.

The flowers of our hardy fruits are of two kinds: (1) anemophilous or wind-pollinated, and (2) entomophilous or insect-pollinated. The unfortunate term "fertilisation" is often applied to the transference of pollen from the anthers to the stigma. To ensure fertilisation it is usually necessary that the pollen and stigma shall be of the same species of plant, and this transference through the agency of wind or insects is termed pollination.

Anemophilous flowers. Among our hardy fruits, anemophily occurs in three natural orders—*Urticaceae* (mulberry), *Cupuliferae* (nuts and chestnut) and *Juglandeae* (walnut).

Insects are rarely attracted to these obscurely coloured flowers, but records show that hive bees will occasionally visit the male flowers of nuts and walnut for the purpose of collecting pollen. The male catkins of chestnut attract a limited number of flies by reason of the aminoid odour, to which certain flies are positively chemotropic.

The researches of Chittenden (5) in England and Lewis and Vincent (19) in America clearly show that there is no ground for supposing that the pollen of apple, pear, plum and cherry is carried by wind. The method adopted was to place vaseline- or glycerine-smeared glass plates at different heights amongst plantations of apple and plum and at varying distances from the trees. At Wisley, the only apple and plum pollen grains collected were found near the remains of insects that had alighted on the plates. Pine pollen was caught in great quantity, the nearest pine trees being about a quarter of a mile away. The American workers only found 67 apple pollen grains on 20 plates. Backhouse (1) carried out some experiments in an English orchard to discover whether wind is capable of pollinating the flowers of a self-fertile variety of plum ("Victoria"). By covering the flowers on one-third of the tree with muslin to exclude insects and yet allow for currents of air to have free play among the blossoms, only one fruit set on the covered portion, whereas a heavy crop resulted on the uncovered portion.

Entomophilous flowers. Among the 16 fruits with entomophilous flowers, 13 belong to the natural order *Rosaceae* and 3 to the *Saxifrageae*.

To consider briefly the several entomophilous fruit flowers, the almond is the first to flower, but the weather is seldom favourable for visits from pollinating insects. Amongst apple and pears, we find none sufficiently self-fertile to be planted in large blocks and still to give good yields. Morello cherries and certain varieties of plums (Yellow Pershore, Egg and Victoria), unlike other varieties of cherry and plum, are self-fertile to such a degree that they may be planted in blocks without interspersing other varieties. The flowers of peach and nectarine, when grown on outside walls, receive very few visits from insects, and it is necessary to pollinate the flowers by hand. Blackberry, loganberry and raspberry flowers are sought after chiefly for pollen, which is collected in large quantities by hive, humble and wild bees and, although self-fertile, better and more perfect fruit is produced when pollinating insects have access to the flowers. Raspberry flowers are less favoured than those of blackberry and loganberry by insect visits. The strawberry is less dependent on visits from insects than any other fruit flower and is partially pollinated by wind. Many American varieties are dioecious, and care has to be taken to see that pistillate forms do not predominate. Black, red and white currants and gooseberry depend entirely on the visits of insects for the transference of the glutinous spherical pollen grains. To exclude insects from the flowers of these plants is to court disaster (Reid (25)), and the practice of fixing a cage made of fine mesh netting over the plantations is to be deprecated. One result of imperfect pollination in black currants is to cause "running-off" (Hatton (13)), that is where only a few flowers on each truss set fruit. The trouble occurs in large plantations when there are an insufficient number of pollinating agents and in small plantations when covered with small mesh netting so that insects are excluded. The introduction of hives of bees reduces the danger.

5. Attractiveness of entomophilous flowers.

The interdependence of flowers and insects cannot be disputed, and entomophilous flowers depend on their colour and fragrance to attract pollinating insects when the pollen is ripe and the stigma is receptive. The attractiveness of colour may often be a difficult factor to explain, for the human appreciation of colour may be different from that of insects. Recent work on this question has been carried out by Lutz (21), who points out that insects are noted for poor vision and a strongly developed sense of smell. Anthophilous insects are known to visit in

large numbers plants with small and inconspicuous flowers, as witness the numbers of wild, humble and hive bees to the flowers of currants and gooseberry. To such flowers they are attracted by a sense of smell. Lutz has shown that pollinating insects can see ultra-violet as well as, or even better, than they can see the rays perceived as light by man. Observations based on about one hundred flowers show that most of the "yellow," many "red" and "blue" flowers are strongly ultra-violet, but that few or no "white" flowers are so.

In order to test how far the floral envelope plays a part in attracting insects, series of experiments have been made in America and England. Lewis and Vincent (19) found that although flowers deprived of their petals were much less attractive, yet several insects pay some visits to blossoms from which the corollas have been removed. Lovell (20), working with pear blossoms, found that once the flowers were deprived of their petals honey bees ceased to visit them for nectar. His two series of experiments may be briefly summarised as follows:

Seven flowers watched for 15 minutes received 8 visits from hive bees. The same 7 flowers deprived of their petals when watched for 15 minutes received no visits and, when watched for a further 15 minutes received 2 visits from hive bees due in part to association (*sic*). Again, one cluster comprising 8 blossoms with petals was watched for 15 minutes and it received 11 visits from hive bees; the other cluster of 8 flowers with the petals removed received no visits.

The author's conclusions are that the bees were guided almost entirely by the presence of petals. That the Wisley experiments do not confirm these results may be seen by consulting Table II.

Observations were carried out on 4 cordon apples, variety "Ecklinville Seedling," two of which had the floral envelope removed from open and unopen flowers on April 29th, 1921, the two remaining cordons being left as controls.

The results as to the insect visitors to normal flowers and to flowers from which the petals were removed are based on observations carried out from April 23rd–May 5th, 1921, over a period of 4 hours 5 minutes at various times of the days in question, generally between 10 a.m. and 6 p.m.

It was found that apple flowers denuded of their petals depended mainly on hive bees, less so on species of *Syrphus* and Anthomyiids, for pollination. Humble bees passed over trees devoid of petals and preferred normal trees situated between and on each side of the abnormal ones. The theory has been put forward that the greater number of hive

Table II.

Insects visiting apple flowers with and without petals.

Species	No. of insects		No. of flowers visited	
	Normal flowers	Denuded flowers	Normal flowers	Denuded flowers
HYMENOPTERA				
Apis mellifica	84	23	1183	432
Bombus agrorum	16	1	152	37
B. lapidarius	2	0	9	0
B. lucorum	10	1	88	1
B. terrestris	5	0	76	0
Andrena fulva	4	3	29	15
A. nana	2	1	15	2
DIPTERA				
Sciarinae	—	Few	Not counted	
Bibio marci	1	0	3	0
Syrphus torvus	25	5	123	11
S. balteatus	21	6	89	36
S. ribesii	1	2	7	13
Eristalis tenax	4	0	11	0
Bombylius major	1	0	1	0
Calliphora erythrocephala ...	3	1	4	2
Anthomyinae	Many	Many	Not counted	
COLEOPTERA				
Phyllopertha horticola ...	1	0	1	0
Adalia bipunctata	2	1	6	2
LEPIDOPTERA				
Pieris napi, ♂	1	0	1	0
Total	183	44	1798	551

bees over other insects on the abnormal flowers is due to the more diligent and systematic search which these insects make when seeking food. The result of another experiment may show that this is not the sole explanation. Artificial apple flowers, anatomically correct, were placed among clusters of apple flowers in one of our orchards, but hive bees were not attracted to them until nectar was placed at the base of the linen petals, after which the bees visited them for the nectar and were attracted to them by the scent of the nectar. The attractive force was odour and not colour. The olfactory sense of humble bees is less keen than the visual and plays a smaller part in the physiology of the insect than in the case of the hive bee. This appreciation for the presence of the floral envelope is shown in Table II, where the numbers of humble bees that visited abnormal flowers are negligible. On the other hand, several individuals

(*Bombus lucorum* and *B. terrestris*) were seen to visit artificial flowers without nectar, the presence of which was necessary before hive bees could be induced to settle on them. *B. lucorum* was found to alight on fallen petals lying beneath apple and plum trees, a fact which shows the important part which the floral envelope plays in attracting these insects.

6. Types of pollination.

The pollination of hardy fruits grown under natural conditions takes place through the agency of wind (anemophily) and anthophilous insects (entomophily).

Peaches and nectarines are usually grown under glass, whilst many varieties of apple, pear, plum and cherry are grown as pot fruit and partially forced under glass. Pollination under these circumstances must be carried out artificially by hand when either a camel-hair brush for experimental work or a hare or rabbit's tail for general purposes is used. Some growers introduce a hive of bees into fruit houses during the blossoming period with complete success so far as pollination is concerned, but the effect on the bees is injurious as will be explained later.

7. Pollinating insects and the transmission of pathogenic organisms.

That there is real danger arising from the visits of insects to fruit flowers is certain and bees and other anthophilous insects have at various times been shown to transmit pathogenic organisms from flower to flower. Barker and Grove (2) have described a bacterial disease of pear blossom, and the conclusions reached were that infection is spread from infected to healthy blossoms through the agency of bees. It was found that healthy flowers were inoculated through the stigma or parts of the flower with which the feet of the insects came into contact. Several bees, which had visited infected flowers, were transferred to sterile Petri dishes and in 50 per cent. of the cases colonies of this particular bacterium were found in the footprints after an interval of three to four days. Bond (3) has observed that whereas the pollen of unopened flowers and those of anemophilous flowers are generally free from microbian infection, that obtained from flowers frequented by hive bees, various species of wild bees and other insects was not. Spore-bearing, gram-negative bacilli together with other bacillary and, in some cases, coccal forms were frequently grown from open entomophilous flowers. It seems probable

that many kinds of open flowers frequented by bees and other insects harbour enormous numbers of organisms, some of which are pathogenic to bees under certain conditions, and the writer suggests that further study of the bacterial flora of flowers would shed light on the diseases to which bees and other insects, besides other animals and even man, are heir. Doidge (6) in her researches on a bacterial blight of pear in South Africa due to *Bacterium nectarophilum* found that the disease organisms were transmitted by bees and other insects. Blossoms covered with paper bags gave a 100 per cent. set, whereas a large percentage of uncovered flowers wilted and fell off. This was attributed to the sheltering effect from high winds and sudden changes in temperature, but it is suggested that it is due mainly to the exclusion of anthophilous insects. Cultures of the causal organism were obtained from bee traces across sterile media in tubes. Other cultures were obtained when the head and thorax of a bee were dropped into sterile plates. Ants are suspected of being concerned in the transmission of the disease.

The case of mite transmission may here be cited. Great numbers of the black currant mite, *Eriophyes ribis* Nal., are found on the foliage and flowers of currants during the migration period, and we have discovered isolated specimens on the legs of *Bombus species* captured whilst visiting the flowers of mite-infested bushes. The danger from the dispersal of mites clinging to the bodies of anthophilous insects must be considered as an ever present menace to mite-free plantations.

8. Insects concerned in the pollination of hardy fruit flowers.

No attempt has been made to arrange the orders, families and genera of plants and insects in strict genealogical order. The families of insects are arranged in accordance with the importance of their members as pollinating agents, whilst the species are in alphabetical order for clarity.

Müller (23, 24) and Knuth (18) give lists of insects visiting the flowers of 14 and 9 different kinds of fruit flowers respectively. Walton (27) lists the number of *Bombus species* found visiting fruit flowers in North Wales. Hatton (13) records the insects taken on black currant flowers at East Malling.

Number of species of insects taken on fruit flowers at Wisley.

	Hymenoptera					Diptera			Coleoptera		Lepidoptera	Neuroptera	Hemiptera		Thysanoptera	Orthoptera	Arachnida
	Apidae	Bombidae	Megachilidae	Andrenidae	Others	Syrphidae	Muscidae	Others	Beetles	Weevils			Heteroptera	Homoptera			
Apple	2	5	1	9	8	14	6	17	15	4	4	2	1	2	1	—	3
Pear	2	3	1	6	4	6	5	6	8	2	—	—	—	—	1	—	3
Quince	2	4	—	3	1	2	2	2	4	3	1	-	-	—	—	—	—
Medlar	1	1	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—
Cherry	2	5	1	4	2	6	2	4	2	1	1	-	-	—	—	—	—
Almond	—	2	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—
Apricot	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—
Peach and nectarine ...	1	2	—	—	—	1	1	—	—	—	—	—	—	—	—	—	—
Plum	2	3	1	8	3	7	3	3	4	—	2	—	—	1	—	1	1
Blackberry ...	2	4	—	3	1	9	1	4	3	—	1	—	—	—	—	1	—
Loganberry ...	2	4	—	4	1	11	1	2	4	—	—	—	1	-	—	—	—
Raspberry ...	2	7	—	9	1	6	—	2	3	—	2	—	1	1	—	—	—
Strawberry ...	2	3	—	2	1	3	1	2	2	—	1	—	—	—	—	—	—
Black currant ...	2	4	—	2	3	2	1	4	1	1	1	—	—	—	—	—	1
Red and white currants	2	3	1	5	5	4	1	7	7	—	1	—	1	1	—	—	1
Gooseberry ...	2	4	—	3	4	2	1	3	4	—	1	—	—	1	—	—	1
Mulberry ...	No records of insect visits																
Nuts, cob and filbert	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chestnut ...	1	—	—	3	1	7	6	9	3	—	—	—	1	—	—	—	—
Walnut	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Local larval habitats of chief pollinating insects of hardy fruit flowers.

Hymenoptera		
Apidae	*Apis* species	In hives; occasionally in hollow trees (Wisley Common)
Bombidae	*Bombus* species	In hedge banks and headlands amongst grass tufts and moss (pinetum and Common)
Megachilidae	*Osmia rufa*	In ground, old wooden fences and palings, gateposts, old bamboo canes, keyholes and snail shells (in and around R.H.S. Gardens)
Andrenidae	*Andrena*, *Halictus* species	In hedge banks, sloping grass banks amongst grass tufts, in gravel paths, in hollow blackberry stems (in and around Gardens and Common)
Vespidae	*Vespa* species	In the ground or suspended from trees and shrubs (in and around Gardens)

Local larval habitats of chief pollinating insects of hardy fruit flowers (continued).

DIPTERA		
Mycetophilidae	*Sciara* species	In decaying vegetable refuse and beneath cow-dung and decayed bark of trees (in pastures and Gardens)
Bibionidae	*Bibio marci*, *Dilophus febrilis*	In living and decaying vegetable matter (in and around Gardens)
Bombylidae	*Bombylius major*	Parasitic upon species of *Andrena* and *Halictus* (which see)
Syrphidae	*Syrphus* species	Predaceous upon various species of Aphides on ornamental flowering shrubs, fruit trees and vegetables (Gardens)
	Platychirus albimanus	In decaying organic matter in damp situations (in Gardens and on Common)
	Rhingia rostrata	In cow-manure (pastures)
	Volucella species	Scavengers in nests of *Bombus* and *Vespa* (which see)
	Eristalis species	In seepage water from pigsties and liquid manure tanks (Gardens)
	Merodon equestris	In narcissus bulbs
Cordyluridae	*Scatophaga stercoraria*	In cow-dung (pastures)
Anthomyidae	Anthomyiids	In living and decaying vegetable matter and manure (Gardens)
Muscidae	*Calliphora erythrocephala*, *Lucilia caesar*	In decaying animal and vegetable matter (Gardens and Common)
COLEOPTERA		
Nitidulidae	*Meligethes aeneus*	Phytophagous on certain *Cruciferae* (headlands and waste land round Gardens)
Coccinellidae	*Adalia bipunctata*, *Coccinella 7-punctata*	Predaceous upon various species of aphides on ornamental flowering shrubs, fruit trees and vegetables (Gardens)

9. Notes on the chief pollinating agents of hardy fruit flowers.

The various orders and families are considered in relation to the importance of their members as pollinating agents.

Order **HYMENOPTERA**.

Fam. **Apidae**. By their more diligent habits, numbers, perceptive qualities and body structure, hive bees play the most important part in the pollination of flowers. They are a governable quantity and are essential for carrying out the work of pollination in large orchards and plantations where it is rarely possible to depend on the work of wild insects. The presence of hive bees in all fruit plantations, especially where large areas are devoted to one kind of fruit, makes cross-pollination doubly sure.

Unfavourable climatic conditions reduce flight, and a long series of observations carried out at Wisley and elsewhere shows that hive bees are affected by low temperatures, even when accompanied by bright sunshine, high percentage of relative humidity in the atmosphere, rain, sleet and snow showers, the presence of cloud, absence of sunshine and high winds (that is above 10 m.p.h.) and cold northerly and easterly winds. The indirect effect of these factors is reduced nectar secretion.

Hive bees work diligently and systematically during bright, sunny, warm weather and will travel to a distance of at least two miles in search of rosaceous flowers. For at least two years, prior to 1921, the hive bees in the neighbourhood of the Wisley fruit plantations were exterminated by disease, yet the crops of apples, pears, plums, currants and gooseberries in 1919 and 1920 were above the average, this being entirely due to the work of humble and other wild bees and flies.

A strong colony of hive bees commences flight at a lower temperature than a weak one, and it is essential for fruitgrowers to obtain females or "swarm" from a healthy stock. Recent work carried out in New Jersey(17) shows that it is necessary to allow one hive for an acre of fruit.

It is not unusual to find hives placed in peach houses in early spring, but this practice is to be deplored for the early activity cannot be maintained, there being an insufficient number of flowers in the open, whilst great numbers of bees die in attempting to escape from the house. The difference in temperature between glasshouse and open air at this period of the year is too great, and the result will be a weakened stock and a great loss in individuals.

During the first warm days in February and March hive bees become active and leave the hive in search of food in the form of pollen and nectar. We have records of bees visiting the male catkins of cob and filbert nuts for pollen.

It has previously been shown that the olfactory sense of honey bees is strongly developed and possibly more so than the visual. This statement whilst agreeing with the conclusions reached by Frisch(10, 11, 12) and Plateau is at variance with those of Wery, who found that bees were as readily attracted to flowers which were completely enclosed in glass globes as those exposed. The observations of Frisch show that when a rich supply of nectar is discovered by a bee, it returns to the hive and executes a series of gyratory movements. Other bees come in contact with it and stroke the abdomen of the dancing bee with their antennae. The bees then leave the hive and search in ever-widening circles for the source of nectar; the number of bees attracted being proportional to the supply. The attractiveness is due to both the scent of the flower and the odour of a volatile substance secreted from a scent-gland situated on the abdomen of the bee and which it has left on the flower. The attraction towards a source of pollen supply is as great, but the character of the "dance" is said to be different from that performed by the nectar collectors.

The number of flowers visited by hive bees in one minute depends on the type of flower (accessibility of nectar and pollen), its condition (partially or fully open) and climatic conditions. During bright, warm sunny weather the rate of visits is accelerated and the average number of apple, pear, plum and cherry flowers visited was 9·6, whilst those of currants works out at 8. The presence of cloud and slight wind slowed the visits down to 6·5 and 5 respectively, and as the weather became stormier their visits ceased altogether.

Two species have been taken on all entomophilous fruit flowers at Wisley since 1921. The common honey bee, *Apis mellifica*, and the Ligurian bee, *A. ligustica*, were frequent visitors to rosaceous flowers and less so to currants and gooseberry, although these flowers depend on visits from anthophilous insects for the transference of the globular and glutinous pollen.

Great danger attends the application of arsenical washes to fruit trees during the period of blossoming. The spraying campaign in orchards must be arranged to

avoid the use of poisonous sprays, particularly those containing arsenic and nicotine, during the flowering period. Attention to this malpractice has been made repeatedly by the Ministry of Agriculture, the South-eastern Agricultural College at Wye and the Royal Horticultural Society through the medium of the horticultural press. The effect on hive bees of spraying fruit trees in blossom with arsenicals has been demonstrated by McIndoo and Demuth(22). It was ascertained that bees work equally well on trees sprayed in full flower as on unsprayed ones, and that they do not fly away from the sprayed orchards to any marked degree if the orchard is well isolated, but they are slightly affected when a small orchard is sprayed in full bloom. Orchard routine may include the application of Bordeaux-lead arsenate spray during the "pink" stage and, as this stage is often of short duration, spraying is continued when many of the blossoms have burst open. It was established by these workers that the minimum fatal dose of arsenic per bee is 0·0004–0·0005 mg.

Fam. **Bombidae.** Nine species of *Bombus* and one species of *Psithyrus*, one of the "cuckoo" bees, have been taken on fruit blossoms. The plantations at Wisley are favoured by their close proximity to open country and pasture, in which humble bees make their nests without fear of disturbance. Where fruit plantations are situated in areas surrounded by building land, such as may be found in suburban areas, and in districts where hedges, with their attendant headlands, are replaced by fences and clean-cut ditches, the number of humble and other wild bees is limited to a number insufficient for the carrying out of pollination.

Fruit flowers belonging to the *Rosaceae* and the *Saxifrageae* were favoured by four species of humble bees, viz. *B. pratorum*, *B. lapidarius*, *B. lucorum* and *B. terrestris*. The last two species are similar in their times of appearance and general activities as witness the number of flowers visited in one minute by each:

(i) *B. lucorum*	16	apple	(ii) *B. terrestris*	18	apple
	16	pear		16	pear
	16	plum		15·6	plum
	14·6	cherry		13	cherry
	12	gooseberry		12	gooseberry

Humble bees, unlike hive bees, are not deterred from their pollinating activities during inclement weather. Observations carried out over a period of five years(28) show that many species of *Bombus* continue to visit various fruit blossoms during (i) high winds and gales, (ii) cold winds, (iii) heavy and continual rain, (iv) snow and hail showers, (v) dull and overcast weather and (vi) from early morning to late evening. During wet and windy weather they are less active and crawl from flower to flower with greater deliberation than during bright sunny weather.

B. pratorum puts in an early appearance during March and April and aestivates in July prior to hibernation. It is a diligent worker, the number of flowers visited in one minute being higher by 1·5–2 than with *B. lucorum*. *B. agrorum* confines itself to the blossoms of apple, cherry, quince and gooseberry flowers, whilst *B. helferanus*, *B. jonellus* and *B. muscorum* have only been taken on raspberry flowers. *B. ruderatus* was occasionally seen on black currant. *B. helferanus* is the last to emerge and does not make an appearance until the end of June and continues throughout July.

Walton(26, 27) remarks that *B. lapidarius* is a late appearing species and is not a frequent visitor to fruit blossoms with the exception of apple and blackberry, but

we have found it visiting the flowers of eight rosaceous plants and also black currant. It is rather an erratic worker and chooses to alight on the uppermost branches of apple, plum, pear and cherry trees and from there working downwards to the lower branches.

Humble bees are more attracted to apple and plum flowers than to pear, for the hawthorn odour of these blossoms, due to trimethylamine, proves somewhat distasteful to them.

The first bees to be observed on early flowering varieties of fruit trees are all females, the neuters appearing later in time to carry on the work of pollination of late flowering varieties of apple, pear, plum and cherry.

Psithyrus quadricolor is associated with *B. pratorum* and *B. jonellus* and was an occasional visitor to the flowers of blackberry and loganberry. Species of "cuckoo-bees" do not possess the pollen-carrying apparatus on the posterior legs, and their visits to flowers are entirely for selfish reasons, while their unmethodical habits make them useless from a pollination standpoint.

Fam. **Megachilidae.** *Osmia rufa*, one of the mason bees, is found abundantly in most fruit-growing areas. Its movements, though slow, do not detract from its importance as a useful agent in the pollination of many fruit flowers. It is a diligent worker, its chief characteristic is its habit of alighting in the middle of rosaceous flowers and crawling over and round the anthers and style. Its nest is formed in hollows in the ground, in old wooden fences and palings, gateposts and keyholes, and even in snail shells.

Fam. **Andrenidae.** This family is an extensive one and 17 species of *Andrena*, 5 speces of *Halictus* and 1 species of *Sphecodes* have been taken on fruit flowers. Members of this family, though known as solitary bees, are often gregarious in their habits, for they choose to make their nests together in a small area and form colonies. They are great pollen collectors, with the exception of species of *Sphecodes* which possess only rudimentary pollen-carrying apparatus.

The species of *Andrena* and *Halictus* vary to a great extent in size, this being found both individually and specifically. Many species are double-brooded, the first brood occurring in April and May and the second in July and August, and one finds the broods often differ slightly in appearance, especially among the males.

Their presence in orchards during the period of blossoming, though desirable, cannot be ensured with the same ease as with hive bees. Most species construct burrows in vertical hedge banks, sloping banks, among grass in headlands and waste land and in gravel paths. The proximity of the orchard to common land and undisturbed areas ensures the presence of many species of this family.

Among the species of *Andrena* that visit fruit flowers, a curious habit has developed, more especially in the spring broods, in the times of their appearance on the blossoms. In the number found and in the increased activity shown, they are strongly represented during the hours from 11 a.m. to 1 p.m. (standard time). Between 1 and 2 p.m. they are torpid and often disappear altogether, but reappear again with lessened activity from 2 to 4 p.m. They prefer to work in flowers during bright sunny weather, and their activities are immediately lessened during the temporary disappearance of the sun behind clouds, at which time they may be found resting in the blossoms or on the ground, resuming their labours on the reappearance of the sun. Very few are found during showery weather, when they either do not venture far from their colonies or crawl sluggishly among the blossoms.

A. albicans and *A. fulva* were most frequently met with on the flowers of hardy fruits, visiting 10 and 7 species respectively. The greatest number of species were taken on apple flowers, whilst raspberry, plum and currants were favoured in their respective order.

The number of individuals in *Halictus* was small, the favourite flowers being blackberry, loganberry and raspberry.

Among the "Sphecode" bees, *S. gibbus* was the only species taken, and confined its attention to apple, plum and cherry flowers.

Fam. **Nomadidae.** Members of this family live as inquilines in the nests of wild bees and are associated more especially with *Andrena* species. The two species taken, one on raspberry and the other on red currant flowers, were uncommon and of little importance as pollinating agents.

Fam. **Eumenidae.** *Odynerus callosus* is the sole species of Solitary True Wasp that has been taken on fruit flowers (apple, pear and plum) at Wisley. Although the females collect small lepidopterous larvae for the purpose of storing their nests as food for the larvae, they occasionally visit flowers and feed on nectar and pollen.

Fam. **Vespidae.** Two species of ground-nesting social wasps, *Vespa rufa* and *V. vulgaris*, and one species of aerial-nesting wasp, *V. sylvestris*, were occasionally seen to visit fruit blossoms during early spring. The females seek nectar and pollen and, on occasion, became predaceous on aphides and lepidopterous larvae that are crawling over the flower clusters. *V. vulgaris*, ♀, was once observed licking the nectar from the leaf nectaries on cherry.

Fam. **Formicidae.** Ants, although frequent visitors to flowers for the purpose of licking nectar, are not useful pollinating agents because they carry but a small amount of pollen on their smooth bodies and do not pass from tree to tree. They are great marauders of unopened pear blossoms, which they frequently bite through in order to reach the nectar.

Fam. **Tenthredinidae.** Sawflies, by reason of the phytophagous habits of their larvae, are unwanted visitors in orchards. The apple sawfly, *Hoplocampa testudinea*, was abundant during the flowering season of 1921, 1922 and 1925. The ♀♀ choose the open flowers for the deposition of their eggs. The insects are extremely active during sunny days and flit from flower to flower with great rapidity.

The gooseberry sawfly, *Pteronus ribesii*, was an occasional visitor to the flowers of currants and gooseberry for nectar, but, unlike the last-mentioned species, the eggs are deposited on the leaves.

Order **DIPTERA.**

Fam. **Cecidomyidae.** A family comprising the gall midges, the presence of which is as undesirable as those of the last family. The raspberry stem gall midge, *Lasioptera rubi*, was a frequenter of apple flowers in 1922. The only other species taken on fruit blossoms was the pear midge, *Contarinia pyrivora*, an ever present pest in many pear orchards but variable in numbers from one year to another.

Fam. **Mycetophilidae.** Members of the family of fungus gnats are classed under the general term "*Sciara species*" in the lists of insects taken on fruit flowers. In numbers they surpass all other insect visitors to apple, pear, plum, cherry, quince, strawberry, currant and gooseberry flowers. Owing to their small size their effectiveness as pollen carriers is overlooked, but the amount of pollen distributed by them

must be considerable. They may be found flitting from flower to flower at all times of the day and during stormy weather when very few insects venture far from their nests.

Fam. **Chironomidae.** Two species of *Chironomus* were taken on apple blossom, but their visits are less purposeful than any other pollinating insect. One species each of *Orthocladius* and *Trichocladius*, however, were not uncommon on the flowers of apple, their habits resembling those of species of *Sciara*.

Fam. **Bibionidae.** The St Mark's fly, *Bibio marci*, was a frequenter of apple flowers and may be classed as a useful pollinating agent.

The most abundant species was *Dilophus febrilis*, which was numerous on apple, pear and cherry. Edwards(9) says of this species that it is "probably one of the most important agents in the fertilisation of fruit blossom."

Fam. **Bombylidae.** *Bombylius major* was an occasional visitor to apple blossom and is strongly heliotropic, for it has never been seen on dull days and ceases its visits during cloudy spells. The trophi are adapted for sucking nectar from tubular flowers such as primrose. The parasitic larvae, according to Chapman(4), are found in the cells of *Andrena labialis* and other species of *Andrena* and *Halictus*.

Fam. **Empidae.** *Empis* species are not truly anthophilous, but may be seen on occasions to suck the nectar of fruit blossom. *E. opaca* and *E. tessellata* were frequent visitors to apple flowers. Their habits are erratic which minimises their usefulness as pollinators.

Fam. **Syrphidae.** This family is strongly represented, many species being found on fruit blossom for the purpose of sucking nectar and feeding on pollen. The larvae may be classified as (i) entomophagous (*Syrphus*), (ii) saprophagous (*Eristalis*, *Platychirus*, *Rhingia* and *Volucella*) and (iii) phytophagous (*Merodon*).

Platychirus albimanus is the smallest Syrphid acting as a pollinator to fruit blossom, and by reason of its numbers may be classified as a useful agent.

Ten species of *Syrphus* are recorded as being attendants on fruit blossom, the most abundant being *S. ribesii* and *S. torvus*, visiting 5 and 11 different species of fruits respectively. Their work is somewhat spasmodic, for they frequently return again and again to the same blossom, after hovering in the air above the flower clusters. As many as 45 flowers are visited in one minute, and their movements from one tree to another are carried out with great rapidity. *S. torvus* is a slower worker and, consequently, a more useful pollinating agent. Apple, blackberry and loganberry flowers attracted great numbers of Syrphids. Three species were frequently found on flowers denuded of their petals (Table II), the odour of nectar proved to be the attractive force. Artificial flowers were not attractive unless nectar was present on them.

Rhingia rostrata is an industrious and frequent worker of apple, pear and blackberry flowers, and *R. campestris* confined itself to cherry blossom. They are fine-weather workers and prefer hot sunny days.

Two species of *Volucella*, *V. bombylans* and *V. pellucens*, were taken on rosaceous flowers. The larvae of the former species are scavengers in the nests of *Bombus*, the latter in the nests of *Vespa*. Their work among fruit blossom is casual and they cannot be classed as useful agents.

The genus *Eristalis* was represented by 5 species, of which 3, viz. *E. arbustorum*, *E. pertinax* and *E. tenax*, were constant attendants on fruit flowers. Their food

consists of nectar and pollen, which they suck up through the proboscis. The excretum of these insects is frequently seen to consist entirely of disintegrated pollen grains. Their habits resemble those of certain species of *Syrphus*, for they work spasmodically and spend a great deal of time hovering over the flower clusters. The resemblance of *E. pertinax* and *E. tenax* to hive bees is marked, both in the way in which they settle on flowers and in the position taken up by the posterior legs when the insects are in flight. Both species have been taken on the flowers of ten species of fruit. *E. arbustorum* stands out as being a more diligent worker. *E. intricarius* visits a limited number of rosaceous flowers and is far less regular in its habits, whilst *E. nemorum* was only taken on blackberry and loganberry flowers. The rat-tailed larvae of these insects are common inhabitants of seepage water from pigsties and liquid manure tanks at Wisley.

The narcissus fly, *Merodon equestris*, confines its attention to apple blossom. It is very agile on the wing and flits from flower to flower, visiting only two or three blossoms on each tree during bright, sunny days.

Fam. **Conopidae**. *Myopa polystigma* was an occasional visitor to apple and cherry flowers. Their habits are sluggish, and they allow themselves to be picked off the blossoms. The larvae are endoparasites of adult bees (*Andrena* and *Bombus*) and wasps (*Vespa*).

Fam. **Cordyluridae**. The common cow-dung fly, *Scatophaga stercoraria*, which is a frequenter of meadows where the eggs are deposited in cow-dung, is found abundantly on fruit blossoms. It resembles *Eristalis* in being partial to the fishy-scented flowers of pear. The tawny-coloured male far outnumbers the dingier-looking female.

Fam. **Anthomyidae**. This very extensive family was represented by several species, but they have not been determined. They are present in large numbers on all fruit blossoms, being partial to rosaceous flowers, from which they can readily obtain nectar. They resemble *Sciara* in that their visits are less purposeful, but their work cannot be ignored for, although small in size, they are indefatigable workers under both calm and stormy conditions.

Fam. **Muscidae**. *Calliphora erythrocephala* may be classed as an important agent in the pollination of apple, pear and plum flowers. Bluebottles frequent these flowers in great abundance, and have been taken on the blossoms of ten other species of fruits. They suck up nectar and are not averse to sucking up rain- and dew-drops on the petals and foliage. Their work is somewhat unstable for they fly away as soon as the tree is approached, but their importance lies in that they are numerous and that they work amongst the blossoms during inclement weather.

Lucilia caesar is less abundant and its erratic habits make it comparatively useless as a pollinating agent.

Musca autumnalis De G. (*corvina* F.) is a common attendant on apple, pear, plum and cherry flowers which it frequents during fine and rainy weather. Its usefulness cannot be questioned.

Fam. **Sarcophagidae**. We do not expect to find anthophilous habits among the pupiparous flesh flies, but *Sarcophaga carnaria* may be frequently seen on the flowers of blackberry, loganberry and raspberry. It is a somewhat heavy insect in the way in which it alights on the blossoms. Its hairy legs become covered with pollen grains as it walks over and around the stamens in its search for nectar.

Order **COLEOPTERA.**

The bodies of most beetles which are found in flowers are devoid of pubescence, and the amount of pollen which they carry from flower to flower is usually negligible. The habit of remaining in one flower for a considerable length of time does not allow them to be classed as useful agents in the pollination of fruit trees.

Fam. **Byturidae.** The raspberry beetle, *Byturus tomentosus*, is a serious pest of raspberry, loganberry and cultivated blackberry, and is an unwanted addition to the already large coleopterous fauna of flowers. The females, besides depositing their ova in the flowers, feed on the floral organs and, occasionally, bite through the stalk so that the blossoms drop off.

Fam. **Coccinellidae.** Aphidivorous ladybirds, of which four species have been taken in fruit flowers, transfer a certain amount of pollen with which their bodies become covered as they move slowly from one flower to another when seeking nectar and aphides. *Adalia bipunctata*, with its wide range of colour variation, and *Coccinella septempunctata*, which is constant in its markings, were taken in ten and eight different fruit flowers respectively. The former, besides satisfying itself with a liquid diet, was observed to feed on the following species of aphides as they crawled over the petals: *Anuraphis roseus* (apple), *A. helichrysi* (plum), *Myzus cerasi* (cherry) and *Amphorphora rubi* (raspberry).

Fam. **Nitidulidae.** A large number of species belonging to this family are anthophilous, and the most numerous of all beetles found in fruit blossom was *Meligethes aeneus*. As many as nine beetles were seen in one pear blossom where they were observed to lick the nectar and devour the floral organs.

Fam. **Scarabaeidae.** The three members of this family, with the possible exception of one (*Aphodius inquinatus*) are useless as pollinating agents. The cockchafer, *Melolontha vulgaris*, and the garden chafer, *Phyllopertha horticola*, are phytophagous, and a great amount of damage to the flowers of apple, quince and other fruit trees is recorded. Several individuals of a dung beetle, *A. inquinatus*, covered with pollen have been observed crawling over the flowers of apple and pear and licking the nectar in them.

Fam. **Elateridae.** The presence of "click-beetles" on flowers is aggravated by the damage they do by eating the petals and anthers. One species, *Limonius cylindricus*, whose larvae is an injurious "wireworm," has frequently been seen to damage the flowers of apple and red currant. During the spring of 1924 many individuals were taken from apple blossom and not, infrequently, two beetles in one flower, the petals of which were speedily reduced to ribbons.

Fam. **Cerambycidae.** The wasp-beetle, *Clytus arietis*, and *Strangalia armata* were the sole representatives of this extensive family of longicorns taken on fruit flowers. Their visits were restricted to blackberry and loganberry, but an occasional wasp-beetle was seen licking the nectar of red currant flowers. They feed on nectar and pollen, but the amount transferred by either is negligible. *S. armata* will often devour the petals and anthers.

Fam. **Chrysomelidae.** *Cassida viridis*, one of the "tortoise" beetles, was an occasional visitor to apple and pear flowers, but it remains too long in one flower

to warrant its inclusion amongst the useful agents. *Lema melanopa*, a beetle injurious to growing cereals, proved to be of little use as an attendant on fruit blossom.

Fam. **Curculionidae.** The four species of weevils recorded from fruit blossoms are all injurious either in the larval and adult stages or in both stages to fruit, and their absence rather than their presence is preferred. The apple blossom weevil, *Anthonomus pomorum*, is a common inhabitant of most orchards and its control is of serious import to fruitgrowers. It has been observed that both sexes, prior to mating, feed by perforating the petals of fully expanded flowers and young foliage. The female oviposits in the unopened blossoms of apple and, to a less degree, pear.

Rhynchites aequatus is a periodic pest of some varieties of apple in orchards in Surrey and Kent. The larval damage is confined to the fruitlets, but the adults perforate the petals of apple and quince and set up a speedy decay of the floral organs.

Two species of *Phyllobius* have been taken on apple blossom. *P. oblongus* was also taken on black currant, whilst *P. pyri*, the more injurious species, committed great havoc by devouring the floral organs and foliage on apple, pear and quince.

Order **LEPIDOPTERA.**

For the sake of convenience the old and more familiar divisions into Rhopalocera and Heterocera are used. Most members of this order possess trophi adapted to a liquid diet. Rosaceous flowers with their shallow corolla do not prove attractive to many butterflies and moths, hence the few records we possess of species visiting fruit blossoms. The fruit plantations at Wisley and elsewhere have been examined at various times during the flowering period of the several fruits between 8 p.m. and 1 a.m., but comparatively few moths have been seen on the flowers. So far as our observations go, there is no evidence to suggest that night-flying moths play any serious part in the pollination of fruit blossoms, although it has been suggested that moths may contribute their quota(8).

Sub-order **Rhopalocera.**

Fam. **Nymphalidae.** *Vanessa io* is the only member of this family that has on occasions been seen imbibing the nectar of apple and plum flowers. Hibernated specimens emerge during warm days in early spring at a time when there are few "butterfly" flowers open. The insect stands on the fully expanded corolla and thrusts its proboscis down into the nectaries, but its body rarely comes into contact with the stamens and pistil, so that the amount of pollen transferred by it is infinitesimal.

Fam. **Pieridae.** The small cabbage white, *Pieris rapae*, and the green-veined white, *P. napi*, have been observed to visit the flowers of apple and strawberry and cherry and blackberry respectively. From the amount of pollen found on their bodies, their presence on fruit flowers is useless to the fruitgrower.

Sub-order **Heterocera.**

Fam. **Hydriomenidae.** One species of *Eupethecia* (the specimens were too much rubbed to determine with certainty) was taken on black currant and gooseberry flowers. The moths were seen flitting among the bushes on dull days. It is not uncommon to find the larvae of the green-pug moth, *Chloroclystis rectangulata*, in apple and quince flowers, which they destroy. They eat the petals and anthers with avidity.

Fam. **Caradrinidae.** *Anarta myrtilli* was observed to suck the nectar from red currant flowers on bright sunny days in 1924.

Order **HEMIPTERA.**

Sub-order **HETEROPTERA.**

Fam. **Pentatomidae.** One of the carnivorous shield-bugs, *Pentatoma rufipes*, was occasionally found crawling over the flowers of loganberry in search of prey, but its slow rate of progress eliminates it as a pollinating agent of any importance.

Fam. **Anthocoridae.** The aphidivorous species, *Anthocorus nemorum*, was taken on apple, raspberry and red currant flowers in and around which it made its way to search for aphides on which it fed.

Sub-order **HOMOPTERA.**

Fam. **Aphididae.** Certain species of aphides with their bodies covered in pollen have at times been taken from fruit blossom. The commonest species being *Anuraphis roseus*, which often clusters on the inside and outside of apple petals. The other species taken as they were crawling over the floral organs were the leaf-curling plum aphis, *Anuraphis helichrysi*, *Amphorophora rubi* on raspberry and *Aphis grossularia* in the flowers of red currant and gooseberry.

Order **THYSANOPTERA.**

Members of this order are grouped with the family *Aphididae* in not possessing sufficiently industrious habits to warrant their inclusion as desirable pollinators. Thrips were found in abundance in apple and pear blossom and, although they distribute a certain amount of pollen as they travel from one flower to another in search of nectar, they derive a great amount of nourishment by puncturing the tissues of the corolla with their piercing mouthparts.

Order **DERMAPTERA.**

Fam. **Forficulidae.** The common earwig, *Forficula auricularia*, is a nocturnal feeder and has been found licking nectar in plum and blackberry flowers. A great amount of damage to plum flowers in 1922 was occasioned by these insects through the destruction of the petals and stamens. Besides their herbivorous habits, they confine their attention to a single cluster of blossom in which they remain until the floral organs are reduced to fragments. Apple blossom is likewise damaged by these insects.

Order **ARANEIDA.**

Spiders visit flowers for no other purpose than the capture of insects as prey. They crawl over the flowers and some species spin webs round the clusters and, in so doing, capture many pollinating insects, chiefly small flies (species of *Chironomus* and *Sciara* and Anthomyiids). Their search continues by night as well as by day, and a small amount of pollen is thus carried by them.

Fam. **Epeiridae.** One of the orb-weavers was well represented on apple, pear and plum blossom, over clusters of which is spun the snare. Individuals were also observed to crawl over the flowers of currants and gooseberry, but no web was spun among the blossoms.

Fam. **Thomasidae**. A species of wolf-spider was seen occasionally to lie in wait inside apple and pear flowers and capture the smaller species of anthophilous insects.

Fam. **Salticidae**. The common British jumping-spider, *Salticus scenicus*, did on occasion wander over apple and pear blossoms, behind the corolla of which it lurked in search of insect prey.

10. Census of chief pollinating agents of hardy fruit at Wisley, Surrey, together with meteorological data (recorded—9 a.m.) of the period during which observations were made.

Apple.

	1920 April 12–May 1 12 h. 40 m.	1921 April 8–May 13 12 h. 25 m.	1922 May 9–26 7 h. 15 m.	1923 April 23–May 13 5 h. 10 m.	1924 May 14–17 1 h. 15 m.
Hymenoptera					
Hive bees	0	85	74	48	15
Humble bees	103	134	55	55	10
Wild bees	10	30	41	16	9
Others	6	10	24	3	1
Diptera					
Hover flies	14	135	46	21	9
Bluebottles	8	11	28	14	3
Others*	45	55	48	31	20
Coleoptera					
Beetles and weevils	9	30	27	1	12
Lepidoptera					
Butterflies and moths	3	4	5	5	1
Plecoptera					
Stone flies	0	0	1	0	0
Mecoptera					
Scorpion flies	0	0	2	1	0
Hemiptera					
Bugs and aphides	2	0	0	0	3
Thysanoptera					
Thrips	2	5	4	7	0
Arachnida					
Spiders	3	3	1	0	1
Mean max. temp. of air (° F.)	56·02	58·95	69·55	60·68	68·00
Mean min. temp. of air	42·74	39·30	46·64	42·90	43·87
Mean min. temp. on grass	34·50	29·52	37·76	34·42	33·50
Mean temp. of soil 1 ft. down	49·59	50·37	56·53	55·49	57·70
No. of ground frosts	7	20	4	8	1
No. of air frosts	0	6	1	2	0
Amount of sunshine (hr.)	72·3	221·1	162·9	129·0	39·2
Amount of rainfall (in.)	1·97	1·45	0·56	1·05	0·02
Prevailing wind	S.W.	N.W. April S.W. May	S.W.	S.W.	S.W.

* Exclusive of *Sciara* species.

PEAR.

	1920 March 25–April 13 2 h. 55 m.	1921 March 7–April 14 8 h. 35 m.	1922 April 21–May 14 10 h. 20 m.	1924 April 25–May 11 1 h. 20 m.
HYMENOPTERA				
Hive bees...	0	41	89	1
Humble bees	14	44	69	8
Wild bees...	0	42	62	0
Others	0	1	4	1
DIPTERA				
Hover flies	6	72	58	1
Bluebottles	5	11	32	6
Others*	41	40	35	4
COLEOPTERA				
Beetles and weevils	5	2	14	0
THYSANOPTERA				
Thrips	3	1	1	4
ARACHNIDA				
Spiders	6	1	2	0
Mean max. temp. of air (° F.) ...	55·40	56·51	57·63	57·20
Mean min. temp. of air	44·33	38·45	34·88	43·53
Mean min. temp. on grass ...	37·43	27·41	27·32	36·50
Mean temp. of soil 1 ft. down ...	48·42	46·13	48·08	51·45
No. of ground frosts	4	26	13	6
No. of air frosts	0	5	5	0
Amount of sunshine (hr.)	47·8	224·5	162·2	76·6
Amount of rainfall (in.)	1·98	1·29	1·28	3·03
Prevailing wind	S.W.	S.W. March N.E. April	N.W.	S.W.

STRAWBERRY. QUINCE.

	1920 May 6–22 2 h. 50 m.	1922 May 20 0 h. 30 m.	1924 May 18–20 0 h. 45 m.
HYMENOPTERA			
Hive bees...	1	5	4
Humble bees	5	8	7
Wild bees...	3	3	0
Others	1	1	0
DIPTERA			
Hover flies	5	4	3
Bluebottles	0	2	2
Others*	9	6	10
COLEOPTERA			
Beetles and weevils	2	6	8
LEPIDOPTERA			
Butterflies and moth larvae ...	2	4	1
Mean max. temp. air (° F.) ...	57·57	75·60	72·95
Mean min. temp. air	42·21	48·90	51·55
Mean min. temp. on grass ...	34·39	36·80	45·10
Mean temp. of soil 1 ft. down ...	53·80	55·80	58·75
No. of ground frosts	7	0	0
No. of air frosts	1	0	0
Amount of sunshine (hr.)... ...	93·6	12·8	11·6
Amount of rainfall (in.)	0·51	0	0·22
Prevailing wind	S.W. partly N.W. partly	S.W. —	E. partly S. partly

* Exclusive of *Sciara* species.

Cherry.

	1922 May 8–9 1 h. 0 m.	1923 April 18–May 14 1 h. 35 m.	1924 May 14 0 h. 30 m.
Hymenoptera			
Hive bees	4	22	5
Humble bees	9	19	3
Wild bees	8	1	0
Others	3	0	1
Diptera			
Hover flies	3	3	1
Bluebottles	5	2	0
Others*	0	2	1
Coleoptera			
Beetles and weevils	2	0	1
Lepidoptera			
Butterflies	1	1	0
Mean max. temp. of air (° F.)	77·90	59·11	73·20
Mean min. temp. of air	45·65	42·21	48·90
Mean min. temp. on grass	35·60	33·85	38·00
Mean temp. of soil 1 ft. down	54·40	52·22	57·30
No. of ground frosts	0	9	0
No. of air frosts	0	2	0
Amount of sunshine (hr.)	22·0	156·5	7·9
Amount of rainfall (in.)	0	1·1	0·01
Prevailing wind	N.W.	S.W.	S.W.

Plum.

	1920 March 25–April 8 3 h. 0 m.	1921 March 10–April 13 3 h. 23 m.	1922 April 21–May 9 9 h. 40 m.	1923 March 26–April 24 8 h. 15 m.
Hymenoptera				
Hive bees	0	15	18	6
Humble bees	34	56	135	83
Wild bees	0	21	46	23
Others	0	1	5	2
Diptera				
Hover flies	1	44	31	13
Bluebottles	2	15	33	18
Others*	5	18	25	22
Coleoptera				
Beetles and weevils	5	2	4	1
Lepidoptera				
Butterflies and moths	1	0	1	0
Hemiptera				
Aphides	0	2	0	0
Orthoptera				
Earwigs	0	0	0	2
Arachnida				
Spiders	1	1	0	0
Mean max. temp. of air (° F.)	54·82	57·35	57·93	55·27
Mean min. temp. of air	43·31	41·63	37·96	40·34
Mean min. temp. on grass	36·29	27·76	29·47	32·24
Mean temp. of soil 1 ft. down	47·87	46·33	48·00	48·50
No. of ground frosts	4	23	11	10
No. of air frosts	0	4	3	3
Amount of sunshine (hr.)	31·0	209·6	131·0	125·0
Amount of rainfall (in.)	1·48	1·28	1·19	1·75
Prevailing wind	S.	S.W. March N.E. April	N.W	N.E.

* Exclusive of *Sciara* species.

	Loganberry.		Raspberry.	
	1923 June 13–July 1 2 h. 35 m.	1924 June 17–19 0 h. 35 m.	1923 June 6–July 1 0 h. 45 m.	1924 June 11–19 1 h. 30 m.
Hymenoptera				
Hive bees	33	4	4	9
Humble bees	24	5	13	19
Wild bees	10	1	6	7
Diptera				
Hover flies	27	13	3	5
Bluebottles	0	1	—	—
Others	0	2	6	4
Coleoptera				
Beetles	2	2	2	1
Lepidoptera				
Moths	—	—	1	1
Hemiptera				
Bugs	0	1	1	—
Aphides	—	—	—	3
Mean max. temp. of air (° F.)	65·24	72·23	64·90	67·34
Mean min. temp. of air	48·52	52·17	48·37	49·77
Mean min. temp. on grass	40·46	43·43	40·82	43·37
Mean temp. of soil 1 ft. down	52·01	63·93	57·50	61·77
No. of ground frosts	1	0	2	0
No. of air frosts	0	0	0	0
Amount of sunshine (hr.)	80·0	31·0	112·1	72·2
Amount of rainfall (in.)	0·26	0·38	0·30	0·96
Prevailing wind	W.	S.W.	W. partly S.W. partly	S.W.

	Black Currant.		
	1920 April 8 0 h. 10 m.	1923 April 5–May 1 3 h. 25 m.	1924 April 23–May 18 2 h. 45 m.
Hymenoptera			
Hive bees	0	0	9
Humble bees	11	46	31
Wild bees	0	3	10
Others	1	3	3
Diptera			
Hover flies	1	5	5
Bluebottles	1	4	3
Others*	1	6	6
Coleoptera			
Beetles and weevils	0	3	1
Lepidoptera			
Moths	0	0	1
Arachnida			
Spiders	0	1	0
Mean max. temp. of air (° F.)	56·00	54·24	59·86
Mean min. temp. of air	47·50	39·83	44·16
Mean min. temp. on grass	46·30	32·08	37·43
Mean temp. of soil 1 ft. down	48·70	48·73	52·93
No. of ground frosts	0	10	5
No. of air frosts	0	3	0
Amount of sunshine (hr.)	0	110·8	125·6
Amount of rainfall (in.)	0·10	2·17	3·52
Prevailing wind	S.W.	N.E.	S.W.

* Exclusive of *Sciara* species.

Red and White Currants.

	1920 April 8 0 h. 10 m.	1922 April 28–May 9 0 h. 50 m.	1923 April 11–26 1 h. 55 m.	1924 April 28–May 18 2 h. 25 m.
Hymenoptera				
Hive bees	0	4	0	2
Humble bees	3	5	10	2
Wild bees	0	3	4	30
Others	1	1	2	2
Diptera				
Hover flies	1	5	5	9
Bluebottles	0	2	10	8
Others*	1	1	15	18
Coleoptera				
Beetles	0	3	0	8
Lepidoptera				
Moths	0	0	0	3
Hemiptera				
Bugs and aphides	0	0	2	2
Arachnida				
Spiders	0	0	2	1
Mean max. temp. of air (° F.)	56·00	61·20	54·00	60·54
Mean min. temp. of air	47·50	38·88	39·04	43·76
Mean min. temp. on grass	46·30	29·59	32·15	36·54
Mean temp. of soil 1 ft. down	48·70	49·05	48·56	53·42
No. of ground frosts	0	7	5	5
No. of air frosts	0	3	1	0
Amount of sunshine (hr.)	0	95·5	70·4	115·7
Amount of rainfall (in.)	0·10	0·49	1·59	2·35
Prevailing wind	S.W.	S.W.	N.E.	S.W.

Gooseberry.

	1920 April 8 0 h. 10 m.	1922 April 28–May 9 0 h. 55 m.	1923 April 9–26 3 h. 20 m.	1924 April 24–May 11 1 h. 0 m.
Hymenoptera				
Hive bees	0	3	0	2
Humble bees	1	4	38	9
Wild bees	0	0	10	1
Others	1	4	4	2
Diptera				
Hover flies	0	3	3	0
Bluebottles	2	3	3	1
Others*	6	9	8	2
Coleoptera				
Beetles and weevils	0	3	1	0
Lepidoptera				
Moths	0	0	0	1
Hemiptera				
Aphides	0	0	1	1
Arachnida				
Spiders	0	0	2	0
Mean max. temp. of air (° F.)	56·00	61·20	53·43	57·30
Mean min. temp. of air	47·50	38·88	38·44	43·00
Mean min. temp. on grass	46·30	29·59	30·34	36·55
Mean temp. of soil 1 ft. down	48·70	49·05	48·12	51·40
No. of ground frosts	0	7	7	4
No. of air frosts	0	3	3	0
Amount of sunshine (hr.)	0	95·5	74·9	77·5
Amount of rainfall (in.)	0·10	0·49	1·91	3·08
Prevailing wind	S.W.	S.W.	N.E.	S.W.

* Exclusive of *Sciara* species.

11. Summary.

It was considered necessary to carry out observations on the insects concerned in the pollination of hardy fruit flowers over long periods in order to arrive at definite conclusions as to what insects may be considered essential for the carrying out of the work.

The result of five years' work has shown the usefulness of the hive bee as a pollinating agent, yet the important work of pollination, under certain conditions, may be carried out entirely by wild insects, principally humble and other wild bees (*Bombus* and *Andrena*) and flies (*Eristalis*, *Syrphus*, *Sciara*, Anthomyiids and *Calliphora*).

The district and the position of orchards govern to a large extent the numbers of wild insects, and the plantations at Wisley are favoured in this respect by their close proximity to open country and pasture land.

The factors, other than the presence of pollinating agents, which influence fruitfulness in orchards are considered.

The transference of pathogenic organisms by anthophilous insects is discussed.

The species of insects taken on the various fruit flowers are listed together with data as to their individual habits and abundance.

A census of the chief pollinating agents of hardy fruit flowers at Wisley is appended and the meteorological data covering the period over which observations were made.

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THE LARVA AND PUPA OF *SCATOPSE FUSCIPES* MG. AND A COMPARISON OF THE KNOWN SPECIES OF SCATOPSID LARVAE

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(With 14 Text-figures.)

During the investigation of insects injurious to stored products in progress at the Imperial College, certain dipterous larvae of the family Scatopsidae were obtained living in green ginger which had been damaged by water. These larvae were accompanied by adult flies which were identified by Mr O. W. Richards as *Scatopse fuscipes* Mg. It was felt that descriptions of the larvae and pupae might be useful in the stored products research, and, as there appeared to be no complete description of Scatopsid larvae, it was suggested that a description of the general morphology of the larva and pupa of *S. fuscipes* should be made.

The material studied was obtained from green ginger at the Metropolitan Wharf, Wapping, in the autumn of 1927. The ginger was unfit for consumption and probably harboured moulds or similar fungi which, together with the *Scatopse* larvae, made it soft, wet and spongy with a marked odour resembling citrus. Larvae and pupae of all stages were found and adult flies were reared.

Previous work on Scatopsid larvae.

The larvae of only two species of Scatopsid have been described previously. *Scatopse notata* was first described, according to the literature cited by Morris (1918), as early as 1776 by Degeer under the name of *Tipula latrinarum*. It was again described by Bouche, 1834, as *Scatopse noir* Geoffr. and by Perris (1847) as *Scatopse punctata* Meig. None of these descriptions was accurate. Dufour (1846) described a larva of *Scatopse nigra* which is thought to be that of *S. fuscipes* Mg. He thought that the larva was amphineustic, not recognising the intermediate abdominal spiracles as such. He mistook the mandibles for maxillae and decided that it had no mandibles. Perris did not make this mistake,

but did not see the maxillae. Both authors describe the labrum. Dufour begins by saying that the body has eleven segments and states later that the eleventh segment consists of two fused, so that there are really twelve. This is the true state of affairs, the dorsal suture being difficult to see.

De Meijere (1917) describes the larva of *Scatopse notata* L. in great detail. He mentions the peculiar labium and hypopharynx but evidently did not see the premandibles and triangular pieces of the labrum. He attaches much importance to the setal pattern, both dorsal and ventral.

H. M. Morris (1918) describes the larva of *S. notata* and compares it with that of *Bibio johannis*. He does not describe the mouth parts other than by a series of drawings which do not agree in detail with my observations.

He lays greatest stress upon the arrangement of the setae. The setal pattern should be of great importance in the classification of these larvae. He describes the pupa.

G. W. Müller (1919) obtained a Scatopsid, *Reichertella femoralis* Mg., from a pupa of *Phora*. This is the only mention of a parasitic Scatopsid.

F. W. Edwards (1925), in his key to the species of *Scatopse*, notes the larval habits of those whose larvae have been recognised. The larvae appear to be saprophagous; they are found on a variety of organic substances all in a state of putrefaction; *S. notata* L. is found commonly in dung, it has also been found in rotten onions (British Museum specimens), and in an old, decaying wasp's nest among dead leaves (Morris, 1918). *Scatopse fuscipes* larvae are recorded as feeding in dung and rotten onions.

Until 1926 nothing seems to have been known of the larvae of species other than *S. fuscipes* and *S. notata*, when M. Tonnoir (1926) described a third species *S. subnitens* Verr. (*S. nigra* Mg.). This species was found under the bark of felled poplars at Hoogstraeten, Antwerp. He gives a general description, emphasising the dorsal setal pattern and the fact that the last larval skin is retained as a covering for the pupa. He does not describe the mouth parts.

It will be seen from the above that the work done on these larvae is meagre and very little is known of the life history of most of them.

SCATOPSE FUSCIPES Mg.

Larva. The general aspect of the larva is very similar to that of *Scatopse notata* as described by Morris and of *S. subnitens* as described by Tonnoir. It is considerably smaller than *S. notata*, measuring only $2\frac{1}{2}$ mm. as compared with 7 mm., the length of *S. notata*.

The head capsule is strongly chitinised. It is bluntly pointed in front, the sides of the head being subparallel. The dorsal surface of the head is almost smooth in the middle but is sculptured laterally. The labrum is marked by several transverse ridges and indeterminate scrolling and bears, about the middle of its length, two small tufts of hairs. The head bears a pair of tufts on each side, the outer tuft consisting of one or two hairs only. The sutures between the frons, clypeus and labrum are not discernible.

The sides of the head capsule are bent under the head, so that they almost meet, leaving only a narrow space between the inner margins; these flaps are the only ventral chitinisation. They taper towards one another and immediately in front of them lies the labium.

There are no eyes.

The antenna consists of three segments. The basal narrow, ring-like and appearing as a swelling on the head; the second also narrow and ring-like but of much smaller circumference; and the third long and finger-like. The second segment bears on its outer or upper surface a spike-like projection similar in form to the third segment, but only about one-third of its width, and about three-quarters of its length. The projection is slightly clavate. The second joint also bears on its upper surface three shorter projections standing side by side. See Fig. 2.

The body of the larva is white and cylindrical, widest in the middle and tapering slightly towards the ends. There is no external difference between the segments of the thorax and the abdomen. There are twelve segments behind the head, the dorsal part of the eleventh covering the twelfth, so that only a short semicircular piece of the twelfth is seen in dorsal view (Fig. 11). In ventral view (Fig. 12) the twelfth is wholly visible and equal in length to the eleventh. The surface of the body is covered with setae which are of various types. There are short stout setae scattered over the surface and longer fine ones which are arranged in an irregular pattern which does not appear to be constant on each segment. In the posterior region of the larva the setae become longer and coarser, while those surrounding the anus are almost bristle-like. The pattern on the first segment, however, is more definite than that on the others and appears to be constant. There is a well-defined transverse row of prominent setae on the proximal border of the seventh to tenth segments on the dorsal surface. The row of setae on the eleventh lies in the middle of the segment and there are no other large setae on this segment (Fig. 1). The sides of the larva are fringed by hairs which increase in length and thickness on the posterior segments.

There are nine pairs of spiracles, borne on the first, and on the fourth to eleventh segments. The spiracles project laterally as small chitinised papillae, wider at the apex than at the base. Those on the eleventh segment are placed on the posterior border of the segment and their papillae are considerably longer than those of the other spiracles. At the apex of the last pair of spiracles is a circular collar bearing a fringe of setae. The trachea can be distinctly seen passing down the papilla and forking as soon as it enters the eleventh segment, one branch apparently entering the twelfth segment (see Figs. 11, 12).

The twelfth segment bears two spiracle-like projections on its caudal border. These projections are chitinised on the dorsal surface only and are fringed with long coarse setae. The anus lies between these projections on the ventral surface. It is longitudinal and is surrounded by a series of membranous lobes which, while protecting the opening, allow of its enlargement when necessary. The anus is further protected by a single row of stout setae which lie outside the anal lobes and project over them.

Mouth-parts. The mouth-parts are well developed.

The labrum (Figs. 3, 4, 5) is narrow in front, the sides sloping away from each other behind. It is sculptured on the dorsal surface, but on the ventral surface the labrum is composed of a trapezoidal, lightly chitinised portion, the narrower margin being posterior. This posterior margin widens out into a semicircular piece—the epipharynx, which is lightly chitinised and is bounded by two half hoops of chitin which almost meet at the apex, this hoop being called by Goetghebuer "la pièce en U" or the U-shaped piece.

The *labrum* is supported by two lateral chitinised plates called by the same author "Les pièces triangulaires." The labrum and epipharynx bear numerous irregularly quadrangular, convex, chitinous swellings, and these in their turn bear numerous long setae projecting posteriorly. The setae project as a fringe around the edge of the epipharynx (Fig. 4). Articulating with the lateral triangular pieces and lying in the membrane are two heavily-chitinised structures, oval and bearing on the margin nearest the epipharynx four large blunt teeth. These are termed by Goetghebuer "premandibles." He describes them in several families of Nematocerous Diptera and in *Scatopse flavicollis* Meigen, as having only three teeth (Figs. 4 and 5).

In *Scatopse fuscipes* they clearly have four teeth and in *S. notata* (Fig. 6) they appear to have five, the fourth tooth being divided into two fine teeth. These premandibles are extraordinarily mobile and, according to Goetghebuer, can move in the antero-posterior plane as well

as in the transverse plane. Edwards (1925) considers that these are primitive structures retained independently in a number of families and lost in the others. They occur in Scatopsidae but not in Bibionidae. This is one of the reasons for separating the Scatopsidae as a family from the Bibionidae.

The mandible. The apical part of the mandible (Figs. 3 and 4) is shaped like a shallow scoop, the base of which at one side is much widened. The scoop is bordered by seven large blunt teeth. Viewed ventrally the wide base of the mandible bears on the apex of its inner margin two teeth, a large and a small. The base of the mandible is hollow, while the outer side below the teeth bears a row of lamellate setae which decrease in size towards the ventral surface. Below these is a single long projection veined like a leaf. At the base of the mandible is a tuft of small setae (Figs. 7 and 8). These mandibles work in the transverse plane and when horizontal conceal the epipharynx.

The maxillae (Fig. 9) overlie the bases of the mandibles in the ventral view. They are more or less membranous and are supported by several chitinised plates. They consist of an outer lobe, which probably represents a one-jointed palp and a wide inner lobe bearing several chitinous teeth on its anterior margin and numerous long setae. The palp carries one circular and one semicircular piece of chitin enclosing numerous small papillae.

The labium (Fig. 10) consists of a semicircular plate, the posterior margin being straight and fringed irregularly with long setae. This plate shows on its ventral surface two crescent-shaped chitinous pieces bearing numerous papillae within their curves. Anterior to the labium is another plate, also semicircular but bearing on each side laterally a chitinous arm, which projects obliquely under the maxillae. This plate shows numerous irregular strongly chitinised areas. Miall and Hammond, when describing the labium of the Chironomous larva, suggest that the anterior plate is the mentum which has partially slipped down behind the posterior plate—the submentum. This seems doubtful. Morris in his description of the larvae of species of Bibio shows a labium consisting of two plates, but does not name them separately. Imms (1925) calls the anterior plate the hypopharynx, and this would seem to be the most probable homology.

The relative positions of the mouth-parts in ventral view is made clear in Fig. 3.

The pupa. (Figs. 13 and 14.)

The larval skin remains as a covering for the pupa. The pupa is widest in the middle and tapered slightly at its extremities, the posterior end being narrower. It is brown, 2 to $2\frac{1}{2}$ mm. long and $\frac{1}{2}$ to $\frac{2}{3}$ mm. wide at its broadest part.

There are six pairs of abdominal spiracles placed laterally on the second to seventh segments. Their papillae are considerably longer than those of the larva and project through the larval skin. On the dorsal surface of the prothorax are a pair of long forked spiracles. The centre of the spiracular structure is strongly chitinised, and in the less chitinised part are numerous laterally-placed chitinous projections which decrease in size towards the apices of the structure. The base of the structure is slightly swollen.

Between the two prothoracic spiracles is a diamond-shaped feebly chitinised area. It is here that the pupal skin begins to rupture before the emergence of the imago, the thorax splitting down the mid-line from this area.

On the ventral surface the antennal sheaths can be seen extending on each side to the base of the wing. The sheaths of the first two pairs of legs meet in the mid-line, the third pair do not meet and all but the apices are hidden by the wing sheaths. The third pair of leg sheaths extend to the posterior margin of the first abdominal segment. The sheaths of the labrum and of the maxillary palps can also be seen, closely attached to the body.

There are eight abdominal segments. The individual segments of the thorax are not clearly indicated.

COMPARISON OF THE LARVAE OF THE THREE SPECIES, *S. FUSCIPES*, Mg., *S. NOTATA*, L. AND *S. SUBNITENS*, VERR.

In the three species the larvae differ greatly in size, *S. fuscipes* measuring $2\frac{1}{2}$ mm., *S. subnitens*, $3\frac{1}{2}$ mm. and *S. notata* 7 mm.

The chief point of difference in the heads of the three species lies in the antennae. The length of the spike borne on the second segment varies in the three species. In *S. fuscipes* the spike is $\frac{3}{4}$ the length of the third segment, in *S. notata* it is about $\frac{1}{3}$ the length and in *S. subnitens* about $\frac{1}{2}$ the length of the third joint. This difference is very marked.

Morris does not describe the mouth-parts of *S. notata* other than by a series of drawings—these drawings differ in several details from the description of *S. notata* which follows:

The mouth-parts are very similar to those of *S. fuscipes* and lateral triangular pieces and premandibles are present. The premandibles of

S. notata have five teeth, the posterior two being very fine. Those of *S. fuscipes* appear to have only four. The labrum is very similar in both species, though the chitinous arms of the hypopharynx in *S. notata* are wider than those of *S. fuscipes*. The mandibles of the two species are similar, as are also the maxillae, the chief difference being in the size. Morris's drawings do not accurately indicate the position of the chitinous plates.

The mouth-parts of *S. subnitens*, as described by Tonnoir, show only that the ventral surface of the labrum bears fine spinules pointing towards the posterior and that the labrum is truncated. It seems possible that they may be similar in essentials to those of *S. fuscipes*.

The only striking differences in the bodies of the larvae lie in the arrangement of the large setae on the dorsal surface of the segments. Those on *S. fuscipes* do not form a definite constant pattern. Those on *S. notata* do, as also do those on *S. subnitens*. The setae on the thorax of the latter species are differently arranged from those on the abdominal segments. There is a median row on all the segments which is doubled on itself in the thoracic segments. The two lateral rows diverge from behind forwards on the thoracic segments and the three lateral rows diverge from before backwards on the abdominal segments.

In *S. notata* the setae are arranged in five longitudinal rows on each segment, except the eleventh, there being little difference in arrangement in any of the segments. On the eleventh there is a single median transverse row of setae which at the sides is continued back to the posterior margin. The anus is protected in *S. notata* by two rows of setae, the long setae around the anal opening and a row of shorter ones on each side. That of *S. fuscipes* is protected by a single row only.

The pupa of *S. fuscipes* differs from that of *S. notata* mainly in size, that of *S. notata* being 4 mm. and that of *S. fuscipes* 2–2½ mm. long. The prothoracic spiracular structures of *S. notata* are shorter and are not forked but bear several short branches.

Tonnoir does not give the length of the pupa of *S. subnitens*, but from his drawing the prothoracic spiracles are apparently similar to those of *S. fuscipes*.

The important differences between the three species seem to lie in the length of the antennal spike and in the setal pattern on the dorsal surface of the larva and to some extent in the shape of the prothoracic stigmata of the pupa.

SUMMARY.

The larvae of *Scatopse fuscipes* were found in decaying green ginger at a London dock in 1927. The necessity for descriptions of larvae occurring in stored products is pointed out.

The previous literature is discussed. General descriptions of the larvae and pupae are given, especial attention being paid to the mouth-parts of the larvae, which have in common with larvae of several Dipterous families, "premandibles."

A short comparison of the larvae of *S. fuscipes*, *S. notata* and *S. subnitens* is made and the most important differences between them are emphasised. No other species have yet been described.

I have to express my indebtedness to Mr O. W. Richards for material and references and to Dr J. W. Munro for his assistance and advice.

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Fig. 1. Dorsal view of larva of *Scatopse fuscipes*: (*a*) antenna; (*b*) head; (*c*) prothoracic spiracle; (*d*) abdominal spiracle; (*e*) spiracle of 11th segment; (*f*) projection of 12th segment.

Fig. 2. Antenna: (*a*) 3rd segment; (*b*) spike; (*c*) 2nd segment; (*d*) 1st segment.

Fig. 3. Ventral view of head: (*a*) mandible; (*b*) labrum; (*c*) lateral piece; (*d*) maxilla; (*e*) premandible; (*f*) epipharynx; (*g*) U-shaped piece; (*h*) hypopharynx; (*i*) labium.

Fig. 4. Labrum ventral view: (*a*) labrum; (*b*) lateral or triangular piece; (*c*) epipharynx; (*d*) U-shaped piece; (*e*) premandible.

Fig. 5. Labrum lateral view: (*a*) labrum; (*b*) lateral or triangular piece; (*c*) epipharynx; (*d*) U-shaped piece; (*e*) premandible.

Fig. 6. Labrum of *S. notata*: (*a*) labrum; (*b*) lateral or triangular piece; (*c*) epipharynx; (*d*) U-shaped piece; (*e*) premandible.

Fig. 7. Right mandible, semi-lateral view.

Fig. 8. Left mandible, ventral view.

Fig. 9. Left maxilla: (*a*) "palp" lobe.

Fig. 10. Labium: (a_1, a_2) hypopharynx, (*b*) labium.

Fig. 11. 11th and 12th segments, dorsal view: (*a*) projection of 12th segment; (*b*) spiracle; (*c*) 12th segment; (*d*) trachea; (*e*) 11th segment.

Fig. 12. 11th and 12th segments, ventral view: (*a*) projection of 12th segment; (*b*) spiracle; (*c*) 12th segment; (*d*) trachea; (*e*) 11th segment; (*f*) anus; (h_1, h_2, h_3) anal lobes.

Fig. 13. Pupa, dorsal view: (*a*) antennal sheath; (*b*) prothoracic spiracle; (*c*) imaginal split; (*d*) abdominal spiracle.

Fig. 14. Pupa, ventral view: (*a*) antennal sheath; (*b*) labrum; (*c*) maxillary palp; (*d*) wing sheath; (*e*) fore leg; (*f*) mid leg; (*g*) hind leg.

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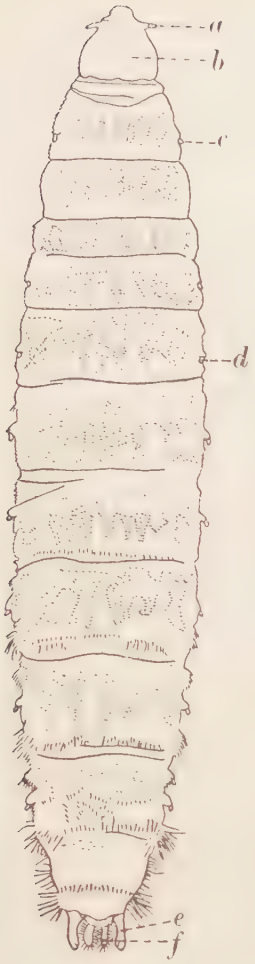
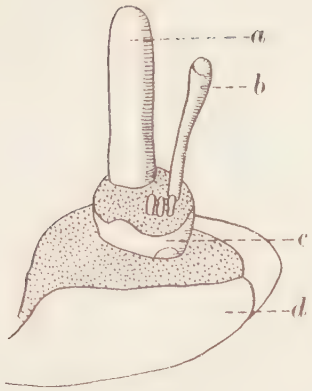


Fig. 1.



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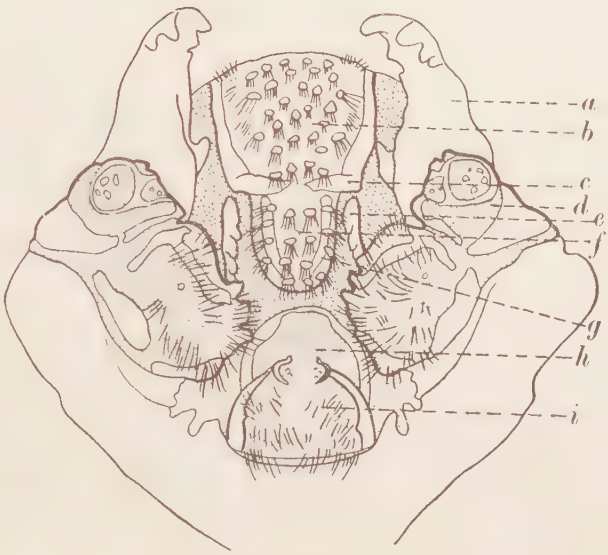

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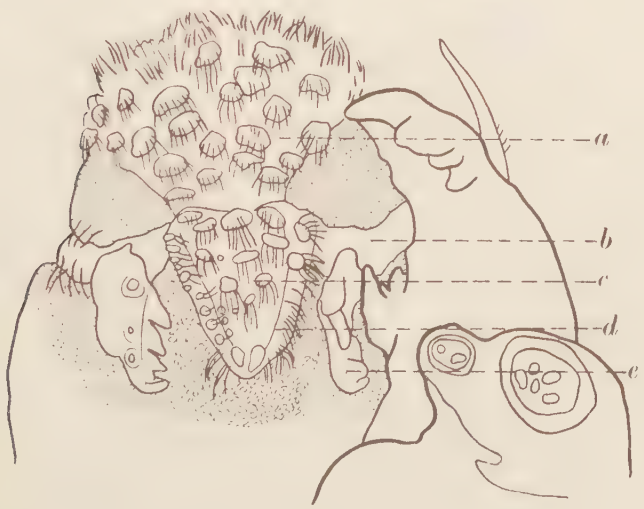

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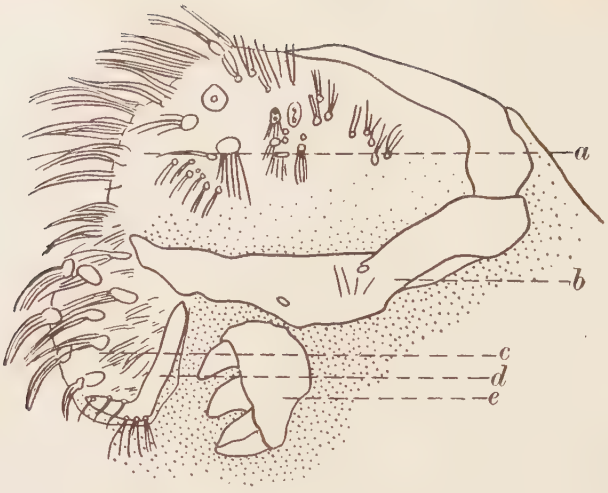

Fig. 5.

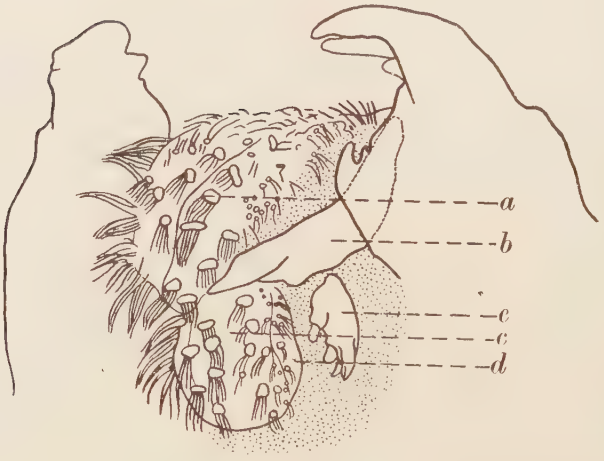

Fig. 6.

Fig. 7.

Fig. 8.

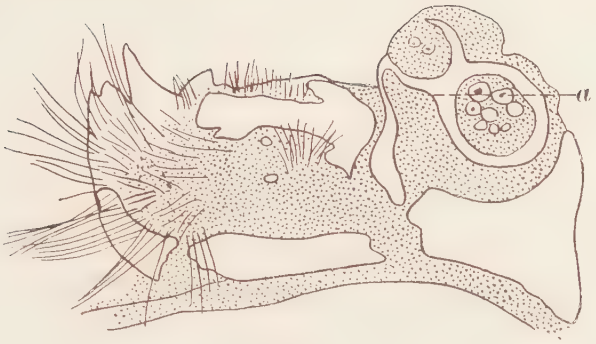

Fig. 9.

Fig. 10.

Fig. 11.

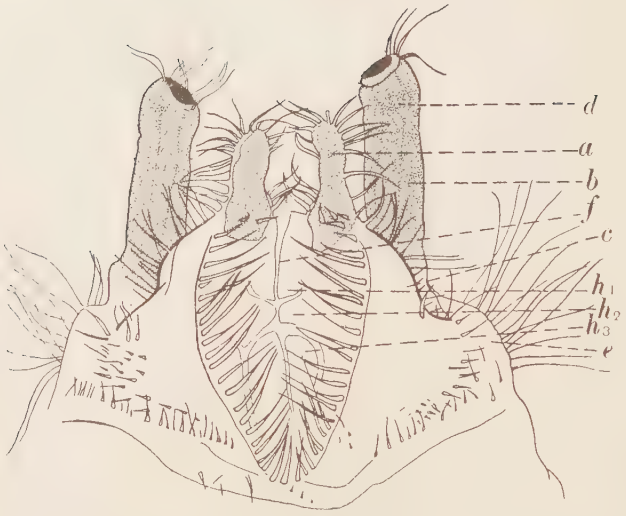

Fig. 12.

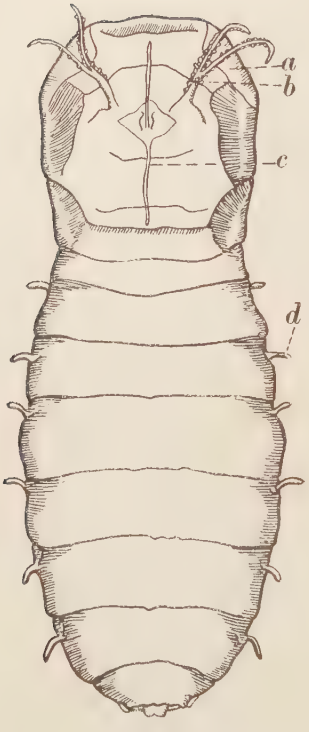

Fig. 13.

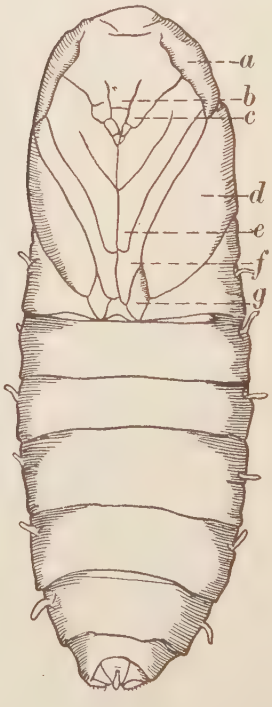

Fig. 14.

REVIEWS

Morphological Variation and the Rate of Growth of Bacteria. By Arthur T. Henrici. Baillière, Tindall and Cox, London, 1928. Pp. xii + 194, with 2 plates, 36 text-figs. and 27 tables. 13*s.* 6*d.* net.

The bacteria as individuals of morphological value have received little recognition during the past generation. This has been due largely to the somewhat blind acceptance of the Cohn-Koch dogma of bacterial constancy and simplicity, the acceptance of the culture rather than the single cell as the individual organism and the concentration of attention upon pure cultural technique rather than upon microscopical examination as a method of research. From time to time, however, students have looked at bacterial individuals through a microscope and seen organisms of unexpected shapes and sizes and possessing apparent methods of reproduction differing from those in the authorised teaching. If they ventured to describe these they were told, gently but firmly, that they were dealing with involution forms, contaminations or merely dirty slides, and if they persisted in their lamentable ways they were ostracised as bacteriological bolsheviks, and it was not infrequently hinted that their work was also suspect on other grounds.

A few students, among whom it is not invidious to exalt the name of Löhnis, have, fortunately for bacteriology, had the courage of their observations, and their work is revolutionising the science.

Professor Henrici goes only part of the way with the newer School of pleomorphists and gives an inadequate account of their researches, but, even so, considers that "they have demonstrated beyond question of a doubt that bacteria do regularly show pronounced morphologic variations, the nature and significance of which must be determined before we can make any real progress towards understanding the fundamental biological problems of the Group."

The general theme of the researches in this monograph is given in the following quotations. "This book makes no pretence of being a treatise on the morphology of bacteria, but is rather a record of personal researches undertaken with the hope that by the 'magic of numbers' some order might be brought out of the chaos which has so far filled that field of bacteriology which has to deal with the form and structure of bacterial cells." "In this work I shall show that, contrary to the orthodox teaching, the cells of bacteria are constantly changing in size and form and structure; but that instead of these changes occurring in a haphazard or meaningless fashion, or instead of being phases in a rather vague and complex life cycle, they occur with great regularity and are governed by relatively simple laws which, after more data have been accumulated and analysed, may probably be very precisely formulated." "My investigations indicate that the growth of bacteria in artificial cultures is governed by the same laws as govern the development of a multicellular organism; that their cells during growth pass through exactly the same sort of a development cycle as the cells of a plant or animal, exhibiting in turn an embryonic form during the period of rapid growth, a mature or differentiated form during the period of slow growth or rest, and a senescent form during the period of death; that in short we may speak of a 'cytomorphosis' in populations of free unicellular organisms differing only in degree from that of multicellular individuals."

The author's general conclusion is represented in the following quotation. "My conclusion that the cell changes occurring in cultures of bacteria are a cytomorphosis of the same kind as that exhibited by the cells of a multicellular organism is arrived at only by analogy. No one can state definitely that the growth and cytomorphosis of a population is governed by laws identical with those which govern the growth

and cytomorphosis of a multicellular plant or animal until it has been discovered what those laws are. But the phenomena so exactly parallel each other, no matter from what angle they are viewed, the analogy is so perfect, that we are justified in accepting this theory as at least a working hypothesis for further investigation."

The organisms used in Professor Henrici's studies were *B. megatherium*, *B. coli*, *V. cholerae* and a diphtheroid bacillus. The author's technique is described in detail and the data are fully recorded in numerous text-figures and tables. The volume closes with eight pages of references and author and subject indices: its format is satisfactory and there are few misprints save the word "diphtheroid" the spelling of which is pleasantly varied.

The book is the first of a new series of Monographs edited by Professors Buchanan, Fred and Waksman dealing with the general agricultural and industrial aspects of microbiology.

WILLIAM B. BRIERLEY.

Die Forleule. Panolis flammea Schiff. Von Dr Hans Sachtleben. Monographien zum Pflanzenschutz. 3. Berlin, Verlag von Julius Springer, 1929. Pp. 160, with 35 text-figs. and a coloured plate. R.M. 15.80 (paper cover).

In the present year there has appeared the beginning of a new series of handy monographs under the editorship of Dr H. Morstatt which is planned to deal with various aspects of plant-protection. The present part, by Dr Hans Sachtleben, is the third of that series and provides a comprehensive account of the biology and means of controlling the Noctuid moth known in Germany as "die Forleule" and in Britain as the "Pine Beauty." Although common in the British Isles, to as far north as Ross-shire, it is not an insect which occasions serious damage to its host-plant (*Pinus sylvestris*). On the continent, however, its status is very different, for it is a forest pest of notable importance and outbreaks of the insect are frequent. In Germany records of outbreaks have been traced back by Dr Sachtleben for 200 years, and in the chronological list of such "Kalamitäten," which he gives, the first record is in 1725 and the most recent attack lasted from 1921 to 1924. During this latter period wide areas of forest in North and East Germany were infested. During 1925 Dr Sachtleben studied the insect in the State forest of Zossen and also in the laboratory in Berlin, and the present monograph is largely based upon those studies. The moths commence to fly at the end of March and the flight is usually over during May. The eggs are laid singly or in rows on the pine needles, and about 500 are laid by a single female. Until their first ecdysis the young larvae cannot feed upon the previous year's pine needles, and consequently the time of appearance of the young needles has an important bearing upon the incidence of outbreaks of the pest.

Dr Sachtleben's full account of the biology of the insect is followed by a very complete discussion of its parasites and hyperparasites. The economy of these natural enemies has been very fully investigated by him in his 1927 memoir (*Arb. Biol. Reichsanst. Land- u. Forstw.* xv, pp. 437–536), and the gist of the information contained in this work is embodied in the present monograph. A very lengthy catalogue of parasitic Hymenoptera and Diptera is given, and it is noteworthy that the decline of the recent outbreak of the caterpillars of this moth and their final disappearance in July 1925 were due to the extensive parasitization which finally got the upper hand.

With regard to control measures, calcium arsenate and sodium fluosilicate are effective as dusts, and in order to ensure the best results they should be applied early in May when the natural stickiness of the shoots holds the insecticides readily.

The Chalcid parasites *Trichogramma evanescens* and *Pteromalus alboannulatus*, which affect the eggs and pupae respectively, are not destroyed by the dusting and consequently these valuable natural controlling agents merit attention. They are easy to maintain by breeding, and the recent work by Flanders in America, on the artificial propagation of the first-mentioned parasite, is referred to in this connection.

In addition to insect enemies, insectivorous birds and mammals also come in for consideration, and the monograph represents a very complete ecological study of the insect from diverse points of view.

Like most German scientific works, it is admirably printed and the coloured plate of the life-history of the insect is well executed: the text-figures are not numerous but will probably be found sufficient for their purpose, while at the end of the work there is a very full bibliography of the insect and its parasites.

A. D. IMMS.

Die Rubenblattwanze Piesma quadrata Fieb. By Joh. Wille. Monographien zum Pflanzenschutz. 2. Berlin, 1929. Pp. 116. R.M. 9.60.

Sugar-beet has been grown commercially in Germany since about 1800, and is now among the more important crops. For 100 years it was not invaded by the Lace Bug *Piesma quadrata* Fieb, although this insect was well known to occur on many of the wild Chenopodiaceae as well as on other plants, *e.g.* in a survey of the pests of the sugar-beet made in 1882 *Piesma* was not recorded, and it has a range in Europe below the 600 m. level from Russia to Great Britain and the Alps to Scandinavia. About the beginning of this century, however, it began to attack the sugar-beet, and, although in some districts it is still chiefly confined to its old hosts, the beet-invading habit has spread over a large area, especially in the middle and east of Germany, and is still spreading. In itself *Piesma* does relatively little damage to the plant, unless the infestation is exceptionally heavy and at the same time the invaded plant young and tender. But its adoption of the new host has been followed by the appearance of a new disease in the sugar-beet, the Krausel Krankheit, or leaf-curl disease, which is a serious menace to the industry in the areas in which it has appeared, and from which it is spreading with considerable rapidity. This is a virus disease, similar in many respects to but not, it seems, identical with the curly-top disease of sugar-beet found in the United States of America. It is carried by *Piesma quadrata*, and, so far as is yet known, by this insect only, and it is transmissible to a number of other plants closely related to the sugar-beet.

In this monograph these points are discussed in detail by Dr Wille, and full details are given of the morphology and life history of the insect, the symptoms of the disease, and the measures to be taken to control its incidence. So far, the most successful method has been to plant a catch-strip of sugar-beet round the fields about two weeks before the main crop is sown. This catch-strip, developing a fortnight before the main crop, is invaded by the *Piesma* that have survived the winter, and, at the time the main crop is beginning to appear, this strip is disinfected thoroughly, and then buried deep in the soil. The results of this procedure are said to be very encouraging.

J. HENDERSON SMITH.

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